PLASMA CATECHOLAMINE CHANGES IN RESPONSE TO EXERCISE IN NORMAL HEALTHY SUBJECTS AND IN POST MYOCARDIAL INFARCATION PATIENTS UNDERGOING REHABILITATION BY EXERCISE THERAPY

by

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ABSTRACT

This study has investigated changes of norepinephrine and epinephrine plasma concentrations, respiratory gas exchange and heart rate response to exercise in healthy, normal adults and post myocardial infarct patients undergoing rehabilitation by exercise therapy.

The acute response to exercise in normals was measured in five healthy trained adults at four different work intensities (900, 1200, 1500, 1800 Kg-m/min) with each exercise lasting nine minutes or until exhaustion. Catecholamine secretion in response to exercise was also measured in serial work tests administered to fourteen post myocardial infarction patients throughout a six month period during which they undertook daily exercise therapy of different types. Exercise took place in the physiotherapy departments of two hospitals or in the Human Performance laboratory at Simon Fraser University with a physician immediately available with emergency equipment at all training and testing sessions. A dc defibrilator and resuscitation equipment, including oxygen and appropriate drugs were on hand.

The original acute measurements on healthy normals established the time relationship of the post exercise plasma catecholamine concentration.

The form of exercise prescribed for the three groups consisted, for five patients (Group I), of cyclic or interval training five days a week for thirty minutes. Cyclic training involves alternate work periods and rest pauses. Another five patients (Group II) trained continuously without pause at a constant work rate for thirty minutes. The

training work rates for both these groups were based upon the previously estimated work capacity expressed in Kilogram-metres/min at a heart rate of 190 beats per min (PWC 190). Cyclic training involved high and low four minute cycles of work interspersed with two minute rest pauses at 60% and 40% of the PWC 190. Four other patients (Group III) exercised in a calisthenic-walk-jog program three times per week for thirty minutes and on two remaining days walked briskly for an equal interval of time. Patient training was upgraded according to the result of the test. After ten weeks, the bicycling group switched to the alternate type of bicycle ergometry and the calisthenic-walk-jog group continued unchanged. After twenty weeks all groups changed to continuous ergometry.

In the five healthy normal males, a progressive increase in the elevation of circulating catecholamines was observed during work and the highest mean values reached by both norepinephrine (NE) and epinephrine (E) occurred just prior to exhaustion. This elevation during exertion was followed by a decline in the catecholamine levels during recovery with a sustained elevation up to six minutes post exercise. The oxygen consumption was found to parallel closely the rise in circulating catecholamines indicating the degree of stress experienced by these subjects with increasing work levels.

In the fourteen post myocardial infarct patients, significant positive changes with training occurred in all the groups in terms of decreased plasma epinephrine at rest and decreased plasma norepinephrine both at rest and during exercise. There was an increase in oxygen consumption concomitant with an increased work capacity and an interaction

between groups in terms of exercise heart rate. Over the twenty-four-week training period, exercise heart rate was found to be fairly constant with increasing work rates. The patients were able to consume more oxygen and do more work at the same heart rate. These results indicate their increase in fitness as a result of the rehabilitative training.

The correspondence of circulating plasma catecholamine levels with induced glycolysis and lipolysis and subsequently with tissue cyclic 3'5'-AMP in working muscle and the provision of substrate for the elevated metabolism accompanying acute physical work are discussed. The dominant role played by the adrenosympathetic system both in the pathogenesis and in the secondary complications of a myocardial infarction is also emphasized.

Elevated levels of circulating catecholamines were observed at rest and after exercise in the fourteen post myocardial infarct patients. These levels decreased after training and approached the levels observed in the normal group of five subjects. It is, therefore, concluded that if sedentary persons are subjected to optimal exercise therapy then lower levels of circulating catecholamines would result and the risk of myocardial infarction would be presented. Since elevated catecholamines and myocardial necrosis have been related, by the same token, if post myocardial infarct patients are subjected to suitable forms of exercise, catecholamine levels will be reduced and the subsequent incidence of secondary infarction prevented, allowing earlier return to work.

TO

MY MOTHER

For Her Understanding

and Moral Support

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CHAPTER 1

INTRODUCTION

This thesis has investigated changes of neurogenic norepinephrine and adrenomedullary epinephrine plasma concentrations, first in the rehabilitation of post myocardial infarct patients under varying methods of exercise and second, during exercise by normal subjects in relation to the relative work levels.

Autonomic nervous system activity may be important in the determination of clinical and physiologic events following myocardial infarction. Since an important question for the physician is whether or not a myocardial infarction necessarily impairs the capacity of the heart for work, it is advisable to examine autonomic nervous system responses to exercise following myocardial infarction using plasma norepinephrine (NE) and epinephrine (E) as markers for the system.

In patients in whom the cardiac reserve is diminished, the normal increase of cardiac output during exercise is attenuated or even abolished (Raab, 1966). It is not known to what extent the sympathetic nervous system is called into play under these circumstances. Diminished activity of this important reserve mechanism could be a factor in the abnormal cardiac response or conversely, an overactive sympathetic nervous system could be a vital compensatory mechanism of such patients. In patients who have suffered coronary heart disease (CHD), it is of interest to assess the activity of the sympathetic nervous system during the stress of muscular exercise, to compare activity in normal subjects and to observe its response to well controlled training.

It has been suggested that in some patients increased physical activity after myocardial infarction is followed by beneficial hemodynamic effects on the heart (Varnauskas, 1966), possibly as a result of increased coronary blood flow (Connor, 1968). This study proposes to investigate the pattern of recovery from myocardial infarction as it is influenced by exercise and reflected by autonomic nervous system responses.

The advent of accurate fluorometric methods for the assay of norepinephrine and epinephrine in blood has stimulated general interest in the pathophysiological significance of these catecholamines. However, thorough investigations of changing patterns of secretion in circulatory plasma catecholamines resulting from regular rehabilitative exercise training has not been carried out. Similarly, the catecholamine concentrations during exercise in relation to the relative work levels or the optimum time of sampling have not been unequivocally established.

PHYSICAL ACTIVITY AND CORONARY HEART DISEASE

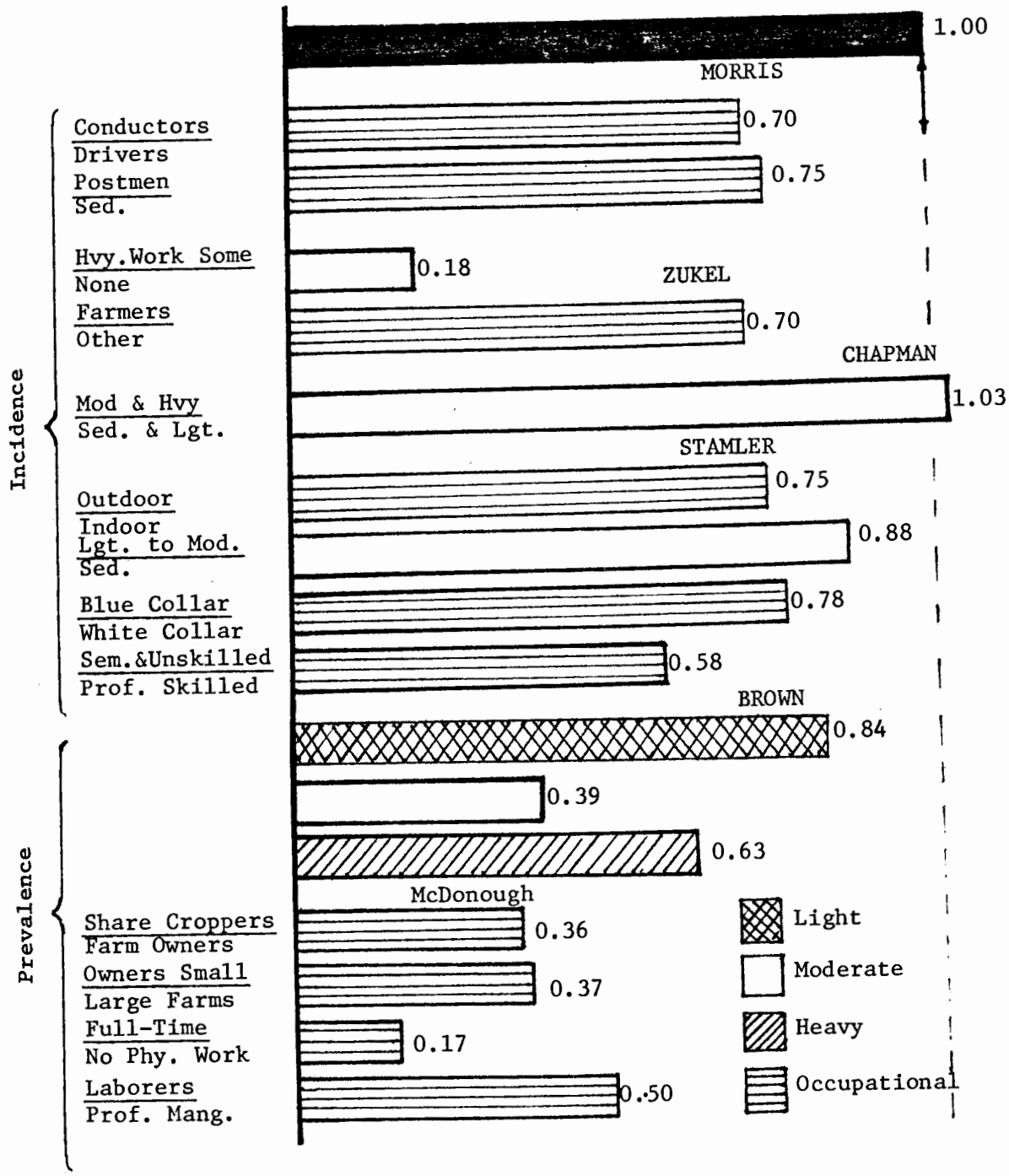
A question arises as to the evidence that regular physical activity per se, compared with a sedentary life, lessens the likelihood of developing coronary atheroscelerosis, coronary thrombosis, myocardial infarction or death from coronary disease.

Information concerning the prevalence and incidence of coronary heart disease related to presumed levels of occupational, and in a few studies, non-occupational physical activity are summarized in Figure 1.

Morris et al., (1953) believed that drivers of London buses were less physically active than conductors and that postal clerks and others

Figure 1. Total Coronary Heart Disease. Persons classified according to presumed levels of physical activity.

The black bar at the top represents the reference (1.00) of total coronary heart disease (CHD) experience of the presumably physically less active group described in the label to the left--the drivers (of London buses) and sedentary postal employees--as compared to the bus conductors or postmen in the study of Morris. The conductors and postmen had 0.70 and 0.75 the incidence of total coronary heart disease experienced by subjects in the respective more sedentary groups. (From Fox and Haskell, 1966).


1.00
MORRIS
Conductors
Drivers
0.70
Postmen
Sed.
0.75
Hvy.Work Some
None
0.18
ZUKEL
Farmers
Other
0.70
CHAPMAN
Mod & Hvy
Sed. & Lgt.
1.03
STAMLER
Outdoor
Indoor
0.75
Lgt. to Mod.
Sed.
0.88
Blue Collar
White Collar
0.78
Sem.&Unskilled
Prof. Skilled
0.58
BROWN
0.84
0.39
0.63
McDonough
Share Croppers
Farm Owners
0.36
Owners Small
Large Farms
0.37
Full-Time
No Phy. Work
0.17
Laborers
Prof. Mang.
0.50
Light
Moderate
Heavy
Occupational
Incidence
Prevalence

whose work was classified as sedentary were less active than postmen who delivered the mail. The incidence of total coronary disease among conductors was 0.70 as frequent as in drivers, and postmen had only 0.75 of the incidence experienced by their more sedentary colleagues. The data of Zukel et al., (1959) indicate that more physically active subjects had only one-fifth of the coronary heart disease found among the less active. The results of Chapman et al., (1957) are an exception to the data of most studies. This is shown in Figure 1 and relates to civil servants in Los Angeles. Stamler et al., (1960) found suggestive but not highly significant differences in their study of the prevalence and incidence of coronary heart disease in strata of the labour force of a Chicago industrial corporation. Brown and colleagues (1957) indicated the prevalence of coronary heart disease among men over age 65 and McDonough et al., (1965) reviewed some marked differences in the biracial population of Evans County, Georgia.

Fox and Haskell (1966) studied the amount of daily activity (hours) associated with coronary heart disease. One or two hours of heavy physical activity per day appear to markedly reduce the incidence of coronary heart disease (Figure 2).

Figure 3 shows studies on myocardial infarction. There is a lower comparative incidence of myocardial infarction than total coronary heart disease in the same groups studied in Figure 1. Brunner and Manelis (1960) studied persons in kibbutzin in Israel and found that the more active group experienced only thirty-three per cent of the myocardial infarction incidence in the sedentary group. The data of Chapman et al., is at variance with the majority of studies in Figure 3. The moderate and heavy workers

Figure 2. Hours of heavy physical activity and incidence of coronary heart disease (CHD) compared to the (national) average. (From Fox and Haskell, 1966).

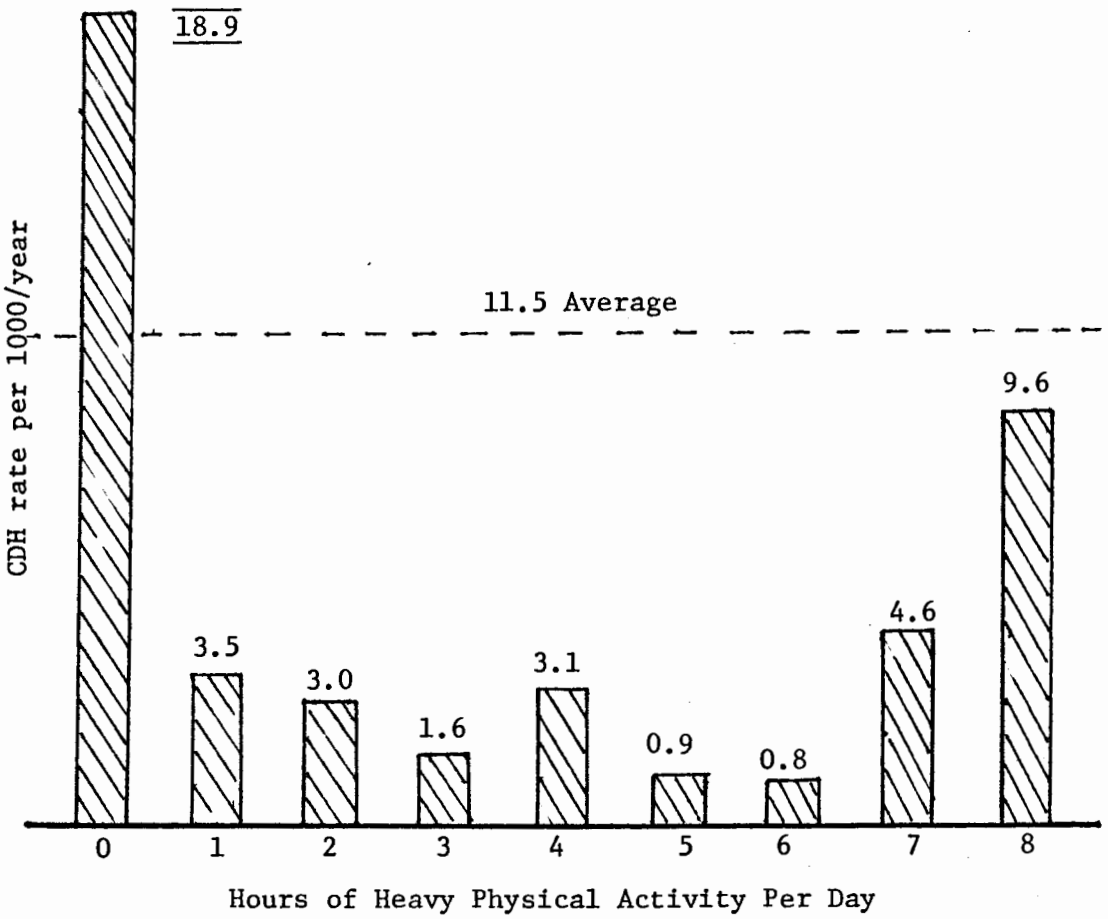
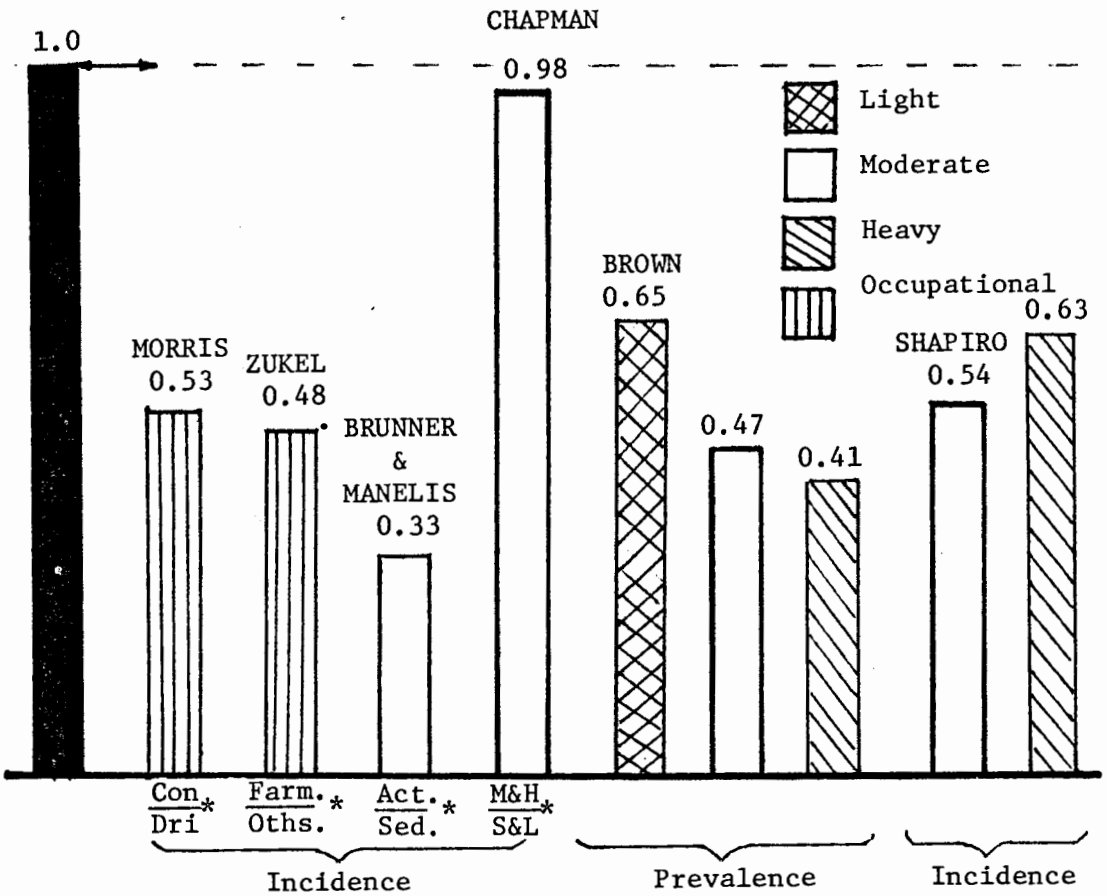

18.9
11.5 Average
3.5
3.0
1.6
3.1
0.9
0.8
4.6
9.6
CDH rate per 1000/year
0
1
2
3
4
5
6
7
8
Hours of Heavy Physical Activity Per Day

Figure 3. Myocardial Infarction. Persons classified according to presumed level of physical activity.

The black bar on the left represents the reference (1.00) of myocardial infarction (MI) experience of the presumably less active group described in the label below, e.g., the drivers (of London buses) compared to the bus conductors in the study of Morris, where conductors had 0.53 the incidence of myocardial infarction than drivers. (From Fox and Haskell 1966).



* Conductors / Drivers Farmers / Others Active / Sed. Mod & Hvy / Sed & Lgt

experienced ninety-eight per cent of the incidence of myocardial infarction experienced by the sedentary group. The report of Shapiro et al., (1965) contains an assessment of non-occupational physical activity which indicates that the heavy workers had a higher incidence of myocardial infarction than the moderate workers. Morris et al., (1966) attribute much of the variance in incidence among busmen to differences in blood pressure and serum cholesterol but do not rule out the significance of physical activity contributing to elevated serum cholesterol and blood pressure. Perhaps, these two parameters are significant indicators of incipient heart disease.

There are indications that in those who are physically more active, both occupationally and otherwise, there is a lower incidence of mortality (Figure 4). Sudden death seems less likely among the presumably more active, and is even less likely than myocardial infarction and coronary heart disease for all classes. Frank et al., (1966) reviewing the Health Insurance Program (H.I.P.) of New York City (Shapiro et al., 1965) and Washington, D. C., postmen (Kahn, 1963) demonstrated that it is only current physical activity which seem to have a beneficial associative relationship.

Morris and Crawford (1958) reviewed 3,800 non-coronary deaths and found that the active individuals had fewer large fibrous patches, fewer small multiple scars and fewer large healed infarcts than the inactive individuals (Figure 5). This trend was definite and consistent. These data reflect individuals sufficiently long in occupations so that the study was considered to be free of bias that would occur as a result of persons shifting to occupations of less physical activity due to incapacity of disease.

Figure 4. Mortality in coronary heart disease (CHD). Persons classified according to presumed level of physical activity.

The black bar at the top represents the reference (1.00 of mortality experience of the presumed physically less active group described in the label to the left—the drivers (of London buses) and sedentary postal employees—compared to the bus conductors or postmen in the study of Morris. The conductors and postmen had 0.46 and 0.50 the incidence of mortality experienced by subjects in the respective more sedentary groups.

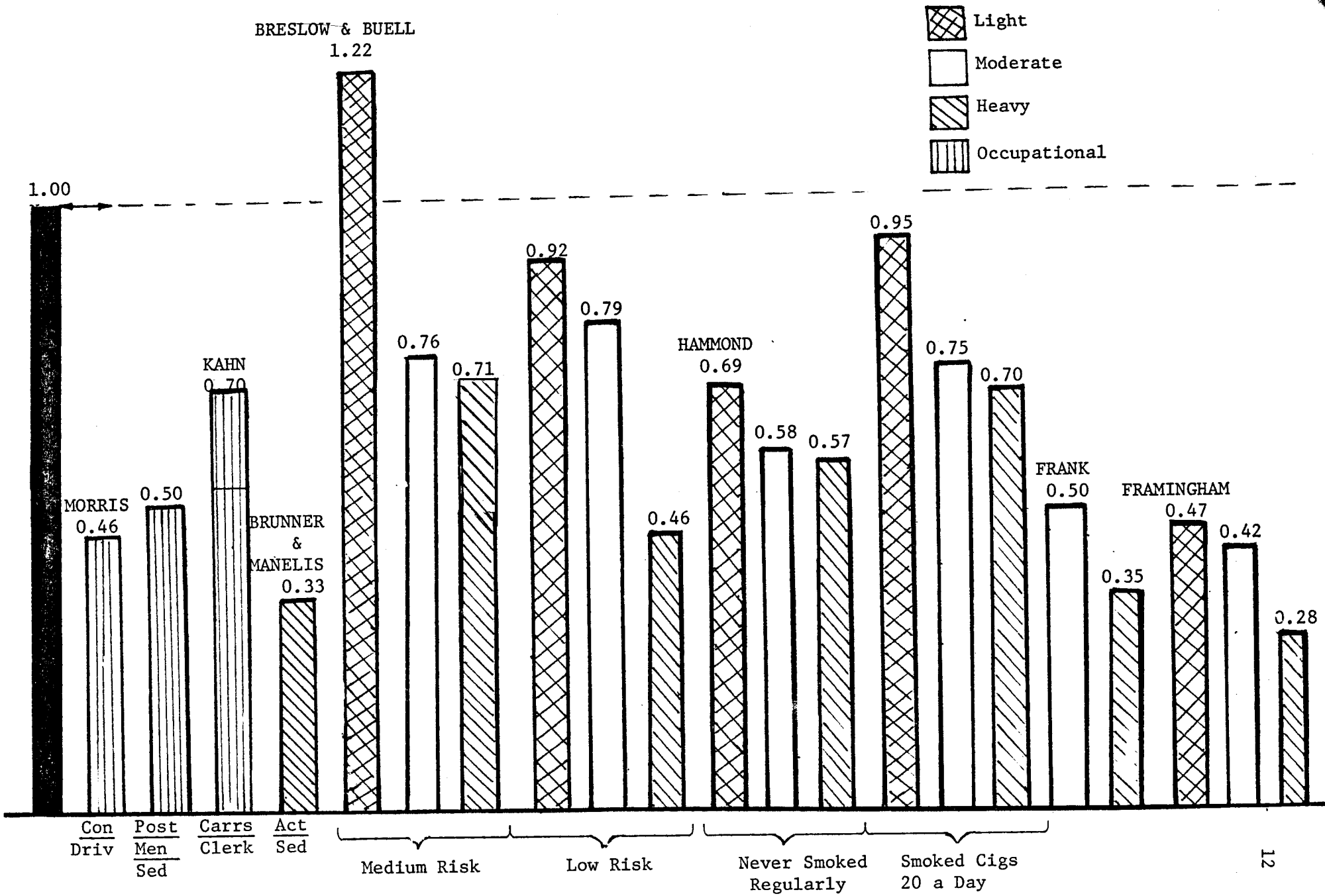
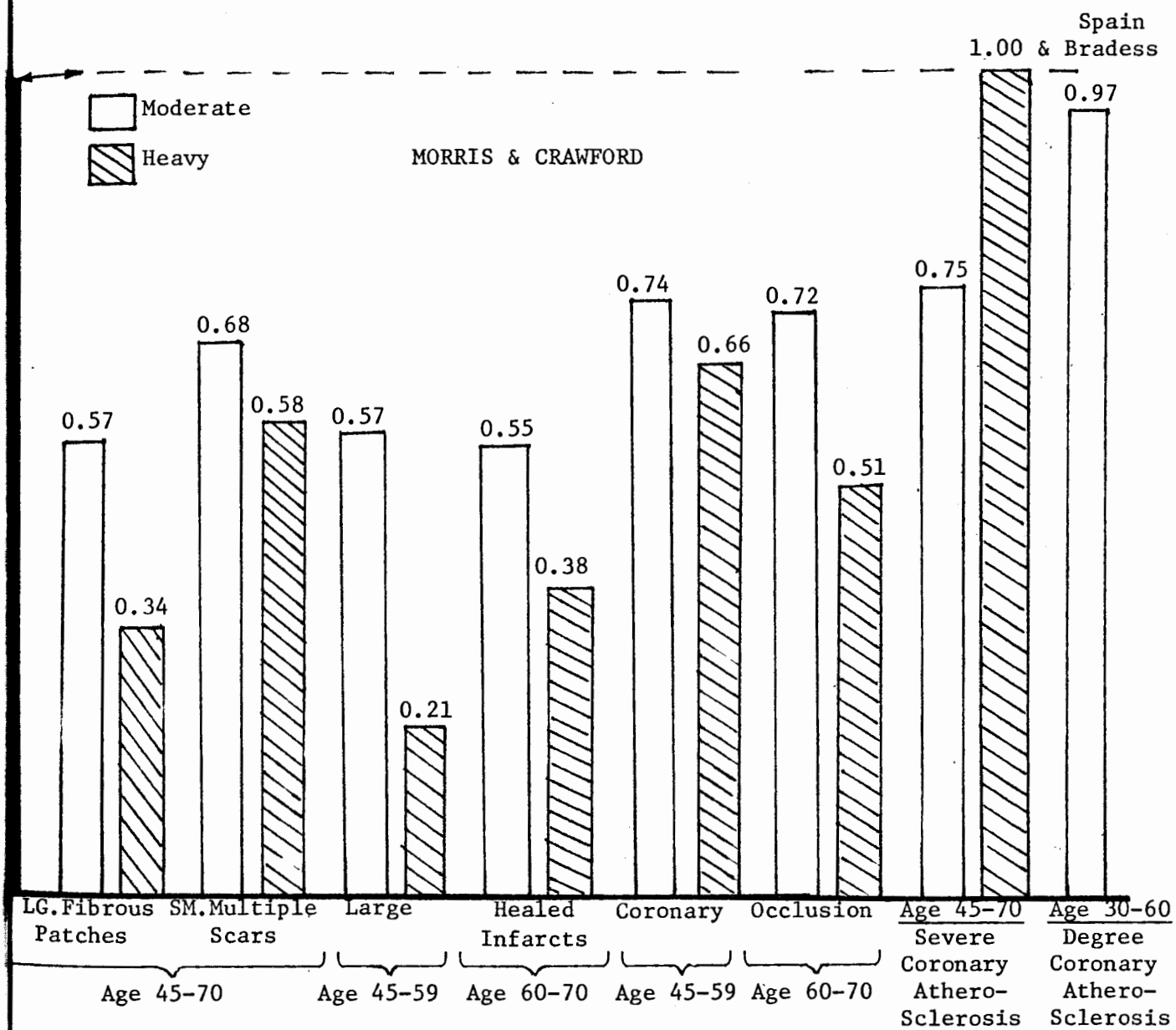

Light
Moderate
Heavy
Occupational
1.00
BRESLOW & BUELL
1.22
MORRIS
0.46
0.50
KAHN
0.70
BRUNNER
&
MANELIS
0.33
0.76
0.71
0.92
0.79
0.46
HAMMOND
0.69
0.58
0.57
0.95
0.75
0.70
FRANK
0.50
0.35
FRAMINGHAM
0.47
0.42
0.28
Con
Driv
Post
Men
Sed
Carrs
Clerk
Act
Sed
Medium Risk
Low Risk
Never Smoked
Regularly
Smoked Cigs
20 a Day

Figure 5. Pathology of Coronary Heart Disease. Persons classified according to presumed levels of physical activity. (From Morris and Crawford 1958).


Spain
1.00 & Bradess
0.97
Moderate
Heavy
MORRIS & CRAWFORD
0.57
0.34
0.68
0.58
0.57
0.21
0.55
0.38
0.74
0.66
0.72
0.51
0.75
LG.Fibrous Patches
SM.Multiple Scars
Large
Healed Infarcts
Coronary
Occlusion
Age 45-70 Severe Coronary Athero-Sclerosis
Age 30-60 Degree Coronary Athero-Sclerosis
Age 45-70
Age 45-59
Age 60-70
Age 45-59
Age 60-70

Mottonen (1970) investigated myocardial infarction and coronary atherosclerosis in autopsy material in Finland. He compared statistically the proportions of pathological changes in intellectual (468 cases) and manual workers (1167 cases) and found that myocardial infarction and coronary thrombosis or occlusion was more often the cause of death among intellectuals than among manual workers, but the difference was not statistically significant. It seems evident, therefore, that the more active individuals are less subject to coronary heart disease, even less to myocardial infarction, and much less to sudden death. The data refers to both occupational and non-occupational physical activity. The major influence of physical activity appears to relate to myocardial infarcts, scars and fibrous patches, less to coronary occlusion and least to coronary atherosclerosis.

Further epidemiological evidence suggests that physically active individuals are less prone to ischemic heart disease than less active individuals (Pomeroy and White, 1958; Brunner, 1966, Pyorala et al., 1967). The evidence so far has encouraged the cautious expression of opinion favouring physical activity as one means of reducing the risk of the development of ischemic heart disease (Davies, Drysdale, and Passmore, 1963; Katz, 1967).

Since an individual cannot be expected to change his job in order to reduce his risk of heart disease and since trends towards mechanization and automation clearly imply that heavy physical work will become increasingly rare, it will be prudent to include increased habitual physical activity in a program to prevent or manage non-acute coronary heart disease.

CARDIAC REHABILITATION -- THE EXTENT OF THE PROBLEM

It has been estimated that there are approximately 18 million people in the United States who have cardiovascular disease of one type or another. On the basis of a national health survey performed by the Public Health Service in 1960, it was estimated that 3.1 million had definite coronary heart disease and 2.4 million had suspected coronary heart disease. A definite myocardial infarction was present in 1.4 million out of 111.1 million adults (Lee and Bryner, 1961). According to the President's Commission on Heart Disease, Cancer and Stroke, there were one-quarter of a million people aged 24 to 64 years who died of heart disease in 1963. The prevalence of heart disease increases as a population ages. Less than 2% of adults between the ages of 18 and 24 years had definite heart disease, but over 40% of adults between 75 and 79 years of age had definite heart disease (report to the President, 1964).

With many of the patients who need cardiac rehabilitation, definitive medical management is often the least challenging aspect for, as has been stated, "the medical treatment itself will actually be the most standardized and simple of the procedures followed in the majority of patients." Rehabilitation of the patient concluding with his reestablishment in the community may often be more difficult because "either we lack the means of physically or psychologically restoring the patient or additionally, the community may not offer him opportunities commensurate with his abilities." (Lee and Bryner, 1961). Unfortunately, the economic losses to families affected by heart disease are large. In one study, it was shown that no more than 22% of cardiac patients in a working population

in New York City were capable of returning or did return to employment within 3 months following the acute myocardial infarction. Further, if work has not been resumed within 10 months, there is little likelihood of subsequent return (Weinblatt et al., 1966). Clark (1959) from the Work Classification Unit in Boston, reported that only two out of 60 patients returned to work within 2 months after infarction. Sharlander's (1964) report on research in the London area was more favourable. Of 212 men less than 60 years of age who had survived a first myocardial infarction without complications, 55% were back at work within 3 months after the onset of the infarction.

EXERCISE IN THE DIAGNOSIS AND PREVENTION OF CORONARY HEART DISEASE

Exercise is a clinical tool that can prove of great value aiding the diagnosis of coronary heart disease. By observing certain precautions, exercise testing is safe, helps clarify clinical sympatomatology and brings out pathologic cardiocirculatory responses not evident at rest. E.C.G. features provide objective support for other clinical findings when present. When E.C.G. findings in otherwise healthy individuals are abnormal, these should be regarded as evidence of potential coronary heart disease, not latent coronary heart disease. The multifactorial etiology of atheroscelerosis involves a family history, diabetes, hypertension, elevated blood cholesterol, cigarette smoking, obesity and coronary involvement. Regular exercise represents the most effective single measure which can be undertaken to prevent coronary heart disease.

Therefore, it is expedient to establish the possible relationship between exercise and cardiovascular health.

EXERCISE IN CARDIAC REHABILITATION

Many physicians have recommended jogging for post coronary patients. A number of studies have confirmed that an exercise program increases exercise tolerance, diminishes left ventricular work for any given load and diminishes the myocardial lactate response. If an individual can exercise after a myocardial infarction without developing cardiac arrhythmias, can augment his heart rate and blood pressure, together with his cardiac output, he is probably better off conditioning by a program of graduated jogging, treadmill running, or bicycling; it is equally important that he is helped to sustain this conditioning by constant reiteration of its importance to him. While it cannot be shown that physical reconditioning prevents subsequent attacks, there is evidence (Kahn, 1963) that there is a lower mortality rate in those who are physically fit.

The optimum time for starting an exercise program after recovery from the myocardial infarction has not been determined. Some experimental programs are introducing grading exercise while the patient is still in the hospital. Certainly by three months after attack he should be ready, and preferably it should be done before he returns to work.

Trained personnel and readily available equipment for emergency use are mandatory in any testing/training program.

CHAPTER II

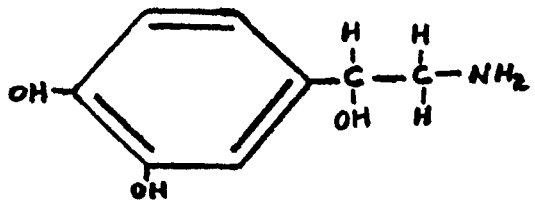
REVIEW OF LITERATURE

THE ROLE OF CATECHOLAMINES IN HEART DISEASE

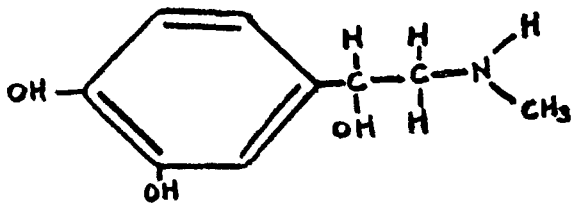
Of all substances synthesized and secreted by mammalian tissues, the catecholamines, norepinephrine and epinephrine, play a major role in regulation of the circulation and pathogenesis of several circulatory diseases.

An augmentation of adrenosympathetic catecholamine action upon the myocardium singly or in combination with impairment of myocardial oxygen supply by structural abnormalities of the coronary vascular system, constitutes one of the most important, if not the most important, cardiopathogenic element in the etiology of degenerative heart disease.

The structure of these catecholamines is simple, consisting of an aromatic group, the catechol nucleus dihydroxy phenol and an aliphatic side chain with an attached amine group.



Norepinephrine



Epinephrine

HISTORY

Serious study of the catecholamines began inauspiciously with the observations of Pelloconi and Foa reported in 1874 and 1884, that extracts of the suprarenal capsules injected into dogs led to general deterioration and death within 24 hours; guinea pigs, rabbits and frogs faired no better.

In 1895, Oliver and Schafer showed that the active principle came from the adrenal medulla and demonstrated the physiological effects on cardiovascular and other organs as we know them today. The subsequent history of the catecholamines is a fascinating story. Intense early activity stimulated by Oliver and Schafer, led severally to the isolation of chemically pure epinephrine by Abel in 1899, to recognition of the similarity between its intravenous administration and direct sympathetic stimulation by Lewandowsky in 1900 and, significantly, to a proposal by Elliott (1904) that adrenaline might be the chemical neurotransmittor of smooth muscle liberated by the nervous impulse.

Following a period of little progress, Barger and Dale in 1910 demonstrated that the sympathetic amine, noradrenaline, reproduced sympathetic effects more faithfully than adrenaline. Loewi and Cannon (1921), independently demonstrated the validity of the concept of neurohumonal transmission. Loewi reported the release of "vagusstoff" and "acceleransstoff" from the frog's heart by stimulation of its autonomic nerves and Cannon and his associates proposed the concept of the sympathins E and I to explain the opposite effects on various organs of the humoral substance released by sympathetic stimulation. Von Euler's identification of

noradrenaline as the adrenergic transmitter, however, did not come until 1946.

Advances were patterned along three pathways. First, neurophysiologists and pharmacologists explored the relationship between the nerve impulse, synaptic transmission, and liberation of transmitter at the nerve ending. Observations by Paton and Zaimis in 1949 on ganglionic blockade, and the subsequent demonstration of alternative methods of blocking adrenergic activity and depleting sympathetic nerves of their catecholamine stores, have provided methods of influencing adrenergic function at both the experimental and clinical levels. This field reached, perhaps, its most interesting point with the recognition, initiated by the observations of von Euler and Hillarp in 1956, of the nerve granules as the storage site of transmitter.

Secondly, an understanding of the biological synthesis of the catecholamines was reached culminating in the demonstration by Blaschko and Maltz, independently, in 1939 of the stepwise production of the catecholamines. These studies led to biochemical control of adrenergic function, at both experimental and clinical levels.

Thirdly, investigation of the concept and activity of receptor sites was advanced. Ahlquist (1948) pioneered these investigations which proposed specific α and β sites receptive to stimulation by catecholamines. Blockade of alpha-receptors was a technique already understood, and specific beta-blockade was subsequently made possible by the separate studies of Powell, Slater, Moran and Perkins in 1958. As a result, a third means of adrenergic control has become an effective and powerful tool for both the experimentalist and clinician.

The 1970 Nobel Prize for Medicine was awarded to three leading investigators in the field of catecholamine research (Axelrod, Katz and von Euler).

UPTAKE, STORAGE AND RELEASE

Norepinephrine arises both from the adrenal medulla and from sympathetic post-ganglionic adrenergic neurons in many tissues (heart, brain, spleen). Epinephrine originates in chromaffin cells in the adrenal medulla.

Thus, norepinephrine is both a hormone and a neuro-transmitter, whereas epinephrine is primarily a hormone. Approximately 15% of the total catecholamine content of the normal adrenal medulla is norepinephrine.

Catecholamines are stored in subcellular membrane-lined granules that protect them from premature metabolic degradation by monamine oxidase (MAO) and other enzymes. Epinephrine resides in chromaffin granules within the adrenergic neuron. The granules contain the catecholamine, a small amount of lipoprotein, and approximately 1 mole of ATP per 4 moles of epinephrine or norepinephrine (Crout, 1968). Norepinephrine may exist in two equilibrated metabolic pools (Axelrod, 1962). The larger is bound within the granule, turns over slowly, and is metabolized within the nerve ending, the smaller pool is unbound, is free in cytoplasm, is susceptible to release by neural stimulation, and turns over rapidly. Granular norepinephrine is replenished both by synthesis and reuptake of unused norepinephrine from the surrounding cytoplasm.

The mechanism by which catecholamines are released is little understood. There is evidence that calcium may be involved especially in the adrenal medulla. Douglas and Rubin (1963) have shown that acetycholine can only release epinephrine and norepinephrine from the perfused cat adrenal medulla in the presence of calcium ions, after perfusion of the gland with calcium-free Locke's solution, the addition of calcium causes the release of catecholamines. In organs other than the adrenal medulla, calcium appears to be required for the release of catecholamines by nervous stimulation. The granules appear to be the principal and possibly the sole source of the catecholamines released from the adrenal medulla.

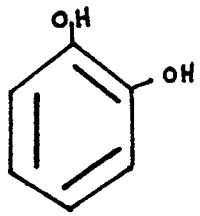
A great number of compounds interfere with the uptake, storage, and release of catecholamines. Examples of these are cocaine, which inhibits uptake, reserpine which inhibits storage and guanethidine which while blocking uptake, also inhibits the release of norepinephrine.

BIOSYNTHESIS

The biosynthetic pathways of epinephrine and norepinephrine are shown in Figure 6. L-tyrosine is found in the circulation in a concentration of 10 to 15 mg/l and is concentrated within cells of the adrenal medulla, sympathetic nerve endings, and brain. It is hydroxylated in mitochondria to form dopa probably by tyrosine hydroxylase.

Dopamine, the first catecholamine synthesized, can diffuse into the circulation, and may be excreted into the urine. It may be metabolized, or can enter the granules in sympathetic nerve endings, chromaffin cells

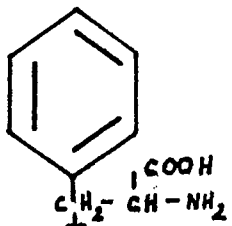
Figure 6. Probable biosynthetic pathways for the production of norepinephrine (NE) and epinephrine (E).



CATECHOL NUCLEUS

TRANSFORMATION

PROBABLE SITE OF ACTION

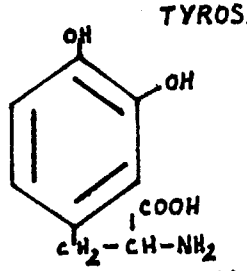


L-TYROSINE

MITOCHONDRIA (ADRENAL MEDULLA, SYMPATHETICALLY INNERVATED TISSUES, BRAIN).

HYDROXYLATION

TYROSINE HYDROXYLASE

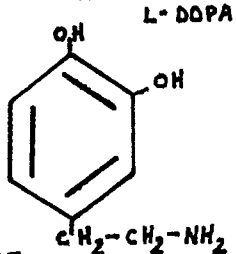


L-DOPA

DECARBOXYLATION

L-DOPA DECARBOXYLASE

SOLUBLE CYTOPLASMIC SAP (MOST TISSUES — BRAIN, KIDNEY, STOMACH, LIVER).

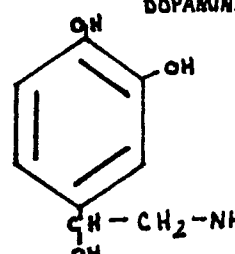


L-DOPAMINE

HYDROXYLATION OF SIDE CHAIN

DOPAMINE β-OXIDASE

CHROMAFFIN GRANULES (ADRENAL MEDULLA, SYMPATHETIC NERVE-ENDINGS).

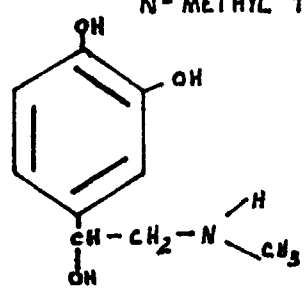


NOREPINEPHRINE

METHYLATION OF SIDE CHAIN

N-METHYL TRANSFERASE

SOLUBLE CYTOPLASMIC SAP OF CHROMAFFIN CELLS.



EPINEPHRINE

and brain cells. It may be stored or hydroxylated by dopamine-β-oxidase to norepinephrine. This is probably the rate-limiting step in the synthesis of the catecholamines and is the final synthetic step in post ganglionic adrenergic neurons and probably in the brain. Only the chromaffin cells found largely in the adrenal medulla contain the necessary enzyme n-methyl transferase, to methylate norepinephrine to epinephrine. Norepinephrine diffuses from the storage granule and is methylated to epinephrine on the surface of the granule or in the cytoplasm of the adrenal medullary cells.

CIRCULATING CATECHOLAMINES

There is a continuous release of small amounts of catecholamines into the circulation and a sharp rise attendent upon enhanced circulatory demands or anxiety states.

The half-life of infused, isotopically labelled catecholamines is extremely short (10 to 30 seconds, Axelrod, et al., 1959), so that most of the epinephrine or norepinephrine utilized by an organ is accumulated during a single circulatory passage and the remainder is recaptured by the sympathetic nerve endings. Organs accumulating catecholamines store them in the granulated vesicles of sympathetic nerve endings rather than in the parenchyma, so that extraction of catecholamines from the blood is proportional to the density of the sympathetic nerve endings. The heart, which is particularly rich in sympathetic nerve endings, contains a high concentration of norepinephrine. Since catecholamines do not diffuse readily across the brain-blood barrier, the brain, rich in catecholamines, does not contribute to the circulating blood content.

PHYSIOLOGIC ACTIONS

Large amounts of catecholamines are secreted in response to emergency stimuli aiding physiological adaptation. A lesser, more sustained secretion, particularly of norepinephrine from sympathetic nerve endings occurs. Norepinephrine is released from granule binding as free norepinephrine, which diffuses from the neuron terminal to a receptor site on the effector cell. A series of intracellular physiologic events is then triggered within the effector cell, leading to the cellular physiologic effect or to a particular response. Adrenergic receptors are cellular components located either on the cell membrane or within the effector cell reacting singly or in combination with epinephrine or norepinephrine. They are viewed as the primary site of catecholamine and other adrenergic mediators, which produce adrenergic effects that are stimulating or inhibiting, depending on the effector organ. Ahlquist (1948) proposed the existence of alpha and beta adrenergic receptors that can mediate the effects of epinephrine, norepinephrine and sympathomimetic drugs. Norepinephrine tends to preferentially stimulate alpha receptors whereas epinephrine stimulates both alpha and beta. Moreover, epinephrine is two to ten times more potent as an alpha stimulator than is norepinephrine. In pharmacologic dosages both catecholamines can stimulate alpha and beta receptors depending on the dosage. Alpha receptor functions are chiefly excitatory and include vasoconstriction on certain vascular beds. Beta receptor functions are mainly inhibitory and include vasodilatation of certain vascular beds,

increased cardiac rate and output. Mixed alpha and beta receptors, however, show both excitatory and inhibitory actions.

CATECHOLAMINES IN HEART FAILURE

The norepinephrine content of the atria and ventricles is decreased significantly in heart failure.

Associated with the myocardial depletion is a state of exaggerated adrenergic activity in the periphery, as indicated by the large increases in norepinephrine content of arterial blood during exercise which is greater than that seen in normal subjects and the excessive urinary excretion of norepinephrine at rest in those patients with congestive heart failure (Chidsey et al., 1965). Thus, in the absence of endogenous myocardial norepinephrine, the circulating catecholamines may provide an important and necessary adrenergic stimulus to the heart, in a supportive role.

CATECHOLAMINES AND ANGINA

Cardiac pain of the anginal type is caused essentially by an acute state of myocardial hypoxia, precipitating a discharge of excessive amounts of cardiac "oxygen-wasting" adrenosympathetic catecholamines in response to impaired coronary vascular dilatability or coronary stenosis.

Therapeutic action against this adrenergic trigger mechanism demands, primarily, a reduction of adrenosympathetic neurosecretary hyperexcitability singly or concomitantly with reduced myocardial metabolic reactivity to catecholamines. This is most effectively done through the

use of beta-receptor-blocking drugs and other direct antiadrenergic medications and cardiac sympathectomy. Indirectly, general metabolic demands may be reduced by drugs controlling thyroid activity.

Exercise training seems to be effective to a certain degree, as a therapeutic method. Various suggestions as to the mechanism of this action have been made including stimulation of coronary collateral formation, (Leon and Bloor, 1968), gradual reduction of cardiac sympathetic tone (Fraser and Chapman, 1954), and promoting of transfer of potassium from contracting skeletal muscles into the myocardium, to replenish hypoxia-induced potassium losses (Rose et al., 1966).

CATECHOLAMINES AND MYOCARDIAL INFARCTION

Coincidence of the mutually aggravating "oxygen-wasting" adrenergic mechanism with oxygen-limiting coronary atherosclerotic conditions compounds the general hypoxic state of the myocardium and appears to precipitate infarction, necrosis and death of a part of the heart muscle itself.

Frequently, these processes are associated with a catecholamine cardiotoxicity and intensifying overproduction of adrenal corticoids. Thus, the adrenosympathetic system plays a dominant role both in the pathogenesis and in the secondary complications of a myocardial infarction. It appears that a most effective and long-acting physiological antihyperadrenergic measure is physical training.

By contrast, the detrimental effects on cardiac function and metabolic demands, by sedentary living, consist chiefly in a loss of adequate parasympathetic and sympatho-inhibitory counter-regulations against adrenergic "oxygen-wasting" metabolism (Raab, 1966).

EFFECTS OF PHYSICAL TRAINING

An extensive review of the physiological effects of muscular exercise is contained in the publication of the Dallas symposium on physical exercise (Chapman, 1967).

In this review, it is suggested that physical training acts to reduce the oxygen consumption of heart muscle and the coronary reserve is increased due to diminution of "oxygen-wasting" sympathelic hyperactivity. Simultaneously, there is an augmentation of parasympathetic tone and sympathetic counter-regulation. In addition, there is a promotion of a more economical heart frequency and of the volume and pressure work of the heart. Persons with trained hearts, therefore, rarely suffer from myocardial infarction.

Lack of training produces the opposite effect always with potentially pathogenic consequences.

The ability of middle-aged individuals to increase their cardiovascular capacity by training has been demonstrated many times. In nine male subjects, between 40-60 years of age who were trained for one hour, three times a week for six months, there was an increase of 15% in maximal oxygen uptake per kgm body weight compared to 1.7% increase for six sedentary controls (Kasch, 1967). Svenson (1967) found that after an eight week period of physical conditioning, ten previously sedentary males, aged 36-56 years, showed increased working capacity and reduced heart rate at a constant work load.

Cumming et al., (1967) studied six boys and six girls between 13-15 years of age undergoing a six day period of training and found a

decline in submaximal pulse rate six beats/min. for a constant load, but no change in measured maximal oxygen uptake. O'Donnell et al., (1967) described a 20 week jogging program of 32 middle-aged and 32 60-year old men. The heart rate during a standard stepping test decreased after four weeks of training by over eight beats/min. and after eight weeks by an additional six beats/min. with no further decrease at 20 weeks. Ekblom et al., (1968) studied eight male subjects during maximal and sub-maximal exercise before and after 16 weeks of hard physical training. The maximal oxygen uptake ($\dot{V}O_2$) increased by 16.2% from 3.15 to 3.68 liters/min. After training, the heart rate, cardiac output, and blood lactate concentrations were lower, the stroke volume was unchanged, and the arteriovenous oxygen difference higher at comparable oxygen uptakes. Hanson et al., (1968) evaluated the effects of a 7 month physical training program on the physiological responses to exercise and work capabilities of 7 middle-aged sedentary men. Testing involved bicycle ergometry and interval treadmill walking, as well as pre- and post-conditioning hemodynamic investigations during 5 levels of treadmill walking up to a 25% grade. Significant alterations of several parameters were observed in the trained state. Resting and exercise bradycardia were marked and the lowest heart rate registered was 49/min. Maximal physical working capacities and $\dot{V}O_{2\ max.}$ increased and there was a relative hypokinesis in submaximal work. Stroke volume was greater at moderate and at the heaviest work loads despite this cardiac minute work reflecting the interaction of decreasing heart rate and increasing stroke volume, was reduced.

EXERCISE AND CARDIAC REHABILITATION

Of great concern in cardiac rehabilitation are the perennial questions:

What proof is there that exercise training programs (physical fitness programs for cardiacs) prolong life, decrease the number or severity of heart attacks, or improve myocardial function?

And if such evidence is available, by what mechanisms does exercise accomplish this?

Further, if exercise is to be prescribed for cardiacs, what type and dose of exercises are needed to produce beneficial effects on the cardiovascular system?

THE PATHOPHYSIOLOGIC BASIS

Exercise may benefit patients with heart disease through:

(1) alteration of cardiac structure

(2) alteration of circulatory function

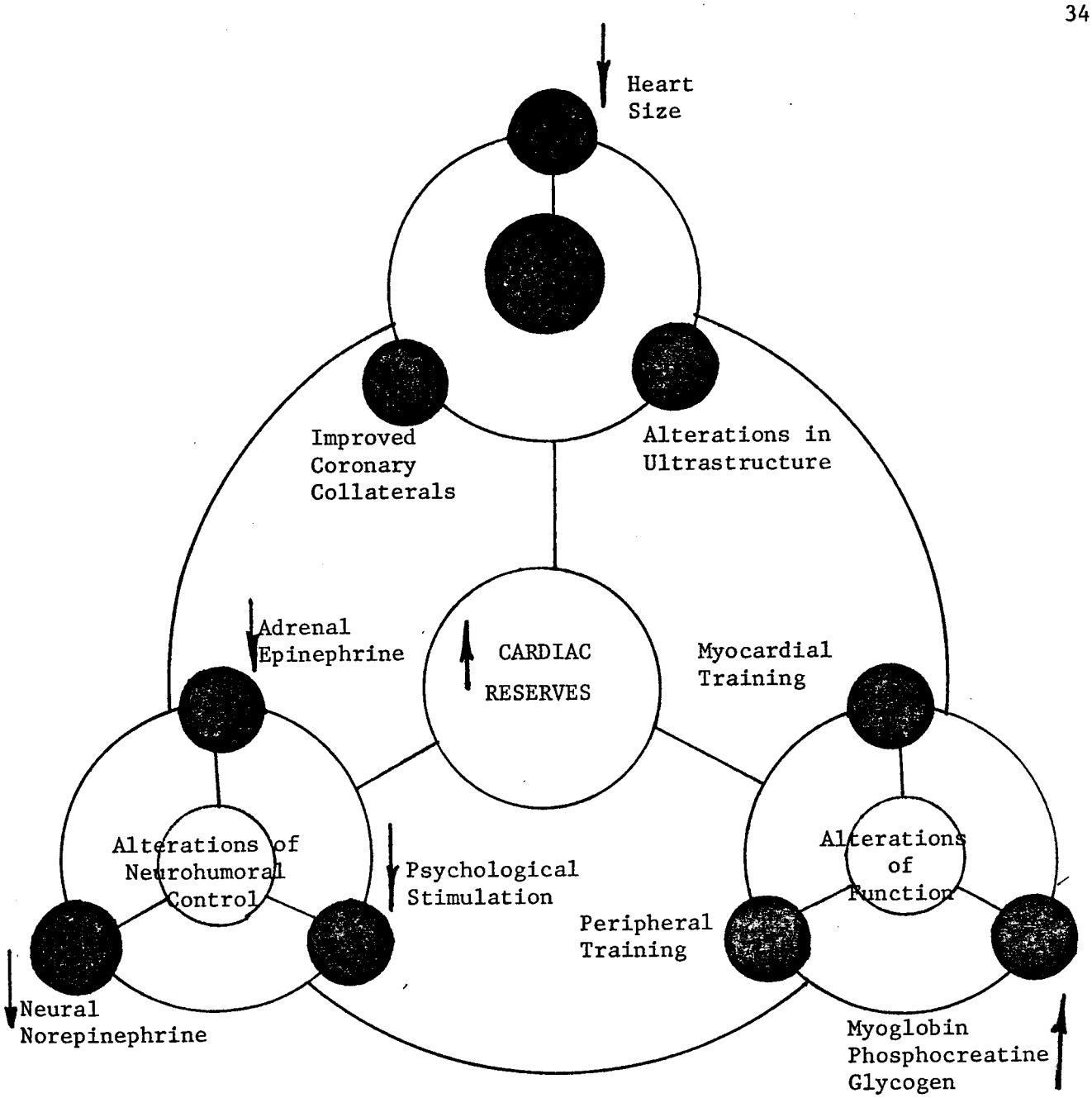
(3) neurohumoral and metabolic changes that result from exercise training (Figure 7).

STRUCTURAL ALTERATION

Of major interest is whether or not exercise training can alter the coronary vasculature. Studies carried out on dogs by Eckstein (1957) showed that regular exercise of these animals on a treadmill, beginning one week after experimentally induced myocardial infarction, resulted in the growth of collateral vessels, in contrast to the lack of growth of

Figure 7. Pathophysiologic basis of exercise training for cardiacs.

Exercise may increase cardiac reserve through alterations of cardiac "structure" or circulatory "function" or through neurohumoral and metabolic changes that result from exercise training. In this diagram upward pointing arrows indicate increased function and downward ones decreased function. (From Zohman and Tobis, 1971).


Heart
Size
Improved
Coronary
Collaterals
Alterations in
Ultrastructure
Adrenal
Epinephrine
CARDIAC
RESERVES
Myocardial
Training
Alterations of
Neurohumoral
Control
Psychological
Stimulation
Peripheral
Training
Alterations
of
Function
Neural
Norepinephrine
Myoglobin
Phosphocreatine
Glycogen

vessels in animals who were not exercised. Years before this, Sutton (1931) found aneurysmal bulging of the ventricles in dogs who were rested only 2-3 days after the creation of the myocardial infarction and then exercised, but in animals rested 6 days and then exercised, aneurysms did not develop and small, well healed scars were seen. Thus it would appear that exercise can improve collateral blood supply without causing aneurysms, even if carried out one week after experimentally induced myocardial infarction in animals. There is the usual difficulty in extrapolating from experiments on animals to man but these findings are encouraging.

Mallory (1939) studied the hearts of 72 patients who died of myocardial infarcts of known duration and found that the smaller infarcts were almost completely healed in 5 weeks, but that the larger ones took 8 weeks or more. On the other hand, he found that infarcts in dogs were well healed in 2 weeks, as in Sutton's (1931) data. He concluded that healing took longer in man than in dogs both because human hearts were larger and the infarcts were therefore larger and because dogs had more collateral circulation. Thomas and Harrison (1944) suggested another explanation of Mallory's findings, i.e., that the greater activity of the dogs might have stimulated the growth of collaterals. The effects of various patterns of activity restriction and free or enforced exercise on rats after myocardial abrasion, simulating infarction, were also studied by Harrison's group. They concluded that mortality is considerably greater when rats are confined in cages post myocardial infarction than when they are allowed to resume normal activities, which they do spontaneously within 3-7 days. Presumably, these findings also may be related

to the stimulation of collateral vessel formation by exercise. In humans, cineangiocardiographic studies of only a few individuals by Kattus (1967) demonstrated the formation of collaterals in angina patients without coronary occlusion who were treated by exercise therapy. Conner (1968) was unable to confirm this increase in collaterals in similar studies on 8 patients with coronary heart disease who exercised in a medically supervised program for a year.

Other studies suggest that exercise training leads to improvement in cardiac patients through alterations of circulatory physiology (Zohman, 1967; Varnauskas, 1966; Sloman, 1965). Exercise training increase the effectiveness with which the circulation adapts to exercise (Cotes, 1965). That is, exercise training results in a more efficient heart with a decreased output and a slower pulse rate for the same work load, oxygen consumption or caloric expenditure. Training also increases the oxygen available to the muscles by stimulating the development of capillaries and increasing the concentration of myoglobin, phosphocreatinine, and glycogen. Aerobic metabolism after training is then able to support more activity than the patient could carry on prior to training. Thus exercise can train both the skeletal and cardiopulmonary musculature. Kryachko et al., (1969) studied the influence of physical exercise on the functional state of the cardiovascular system and its role in the rehabilitation of patients with coronary disease in 100 patients who received sustained treatment in hospital, in the convalescent department and in the outpatient clinic, as well as prescribed physical exercise. Twenty patients who received no physical exercises served as controls. They found that

timely, regular and sustained use of physical exercise combined with drug therapy of patients with coronary disease helps to improve the myocardial function and the development of compensatory mechanisms of circulation. It enhances the efficacy of treatment and contributes to more rapid rehabilitation.

Neurohumoral and metabolic factors also influence cardiac function and cardiac reserves, and these, too, may be influenced by exercise. Exercise training programs can, seemingly, reverse some of the detrimental effects of unaccustomed stress by stimulating cardioprotective resistance, that is, by decreasing the outpouring of catecholamines during activity (Raab, 1966). Catecholamines (adrenomedullary epinephrine and neurogenic norepinephrine) are "oxygen-wasting" in that they require myocardial oxygen for their metabolism, thus depriving the cells of the full amount of oxygen delivered to them by the coronary circulation. Ischemic loss of myocardial glycogen and potassium, changes of metabolism, and a decline in myocardial contractility may occur in the presence of circulating catecholamines with ultimate hypoxic damage or even focal necrosis.

Raab et al., (1966) have provided evidence for a reduction by habitual physical exercise of the exaggerated cardiac sympathetic tone and reactivity which results from sedentary living and which endangers myocardial oxygen economy, especially in the presence of coronary atheroscelerosis. Thus, exercise training may be beneficial to the cardiac by decreasing myocardial oxygen demands. In addition to these possible benefits of exercise training programs to peripheral musculature

or coronary vessels, there is the widely acknowledged clinical observation of the sense of well-being and improved physical performance in daily activities experienced by those participating in such programs.

Hellerstein (1968) reports subjective improvement with a state of well-being in over 90% of subjects who participated in such a reconditioning program. Although it is admittedly difficult to obtain valid comparative data on long-term survival after infarction, Hellerstein (1966) reported that in 697 patient-years there were 11 deaths from coronary disease of men on the program. He concluded that the incidence is 5 deaths per 100 patients per year, so that 35 might have been expected to occur without the fitness program. Brunner (1968) in Israel reports 2 deaths and 4 second infarcts in 64 patients during the first year on the reconditioning program, compared to 7 deaths, 9 recurrent infarcts, 10 congestive failures, and 30 angina cases among an equal number of myocardial infarct patients who did not participate in the program.

Sloman and associates (1965) found an increase in the physical working capacity of trained cardiac patients; Rechnitzer et al., (1965), observed increased muscular endurance and Varnauskas, Bergman, Houk and Björntarp (1966), improved exercise tolerance and reduced left ventricular work of similarly trained cardiac patients. In addition, electrocardiographic improvement has been noted in trained cardiac patients (Hellerstein et al., 1967). Exercise has been claimed to reduce the blood cholesterol, lower the blood pressure, diminish skinfold thickness, reduce obesity and increase glucose tolerance (Hickie, 1968). There is general

agreement that exercise programmes do reduce some, if not all, plasma lipids (Montoye et al., 1959; Hollozy et al., 1964; Carlson and Mossfeldt, 1964; Shane, 1966; Campbell, 1965; Naughton and McCoy, 1966).

Training experiments in dogs, established that physical conditioning can be achieved in the presence of acute myocardial infarction (Kaplinsky et al., 1968) and that the exercise program is not harmful. The experiment was performed in two groups of dogs which had their coronary arteries ligated to produce infarction of 15-30% of the left ventricle. All animals were then examined during exercise between the 3rd and 6th day after the ligation. Thereafter, the animals were divided randomly into a trained and a control group. The trained group was exercised on a treadmill for 30 minutes, twice daily, six days a week for a period of five weeks after the coronary occlusion. After training, these dogs had a slower heart rate both at rest and during exercise and also a lower cardiac output. The control dogs showed significantly greater rises in lactate and plasma catecholamines during exercise than the trained animals. No harmful effects of training developed. All trained animals established collateral channels.

A training program for patients with hypokinetic circulation has been tried with good results in patients with healed infarctions (Frick and Katila, 1968; Rechnitzer et al., 1967a; Rechnitzer et al., 1967b and McPherson et al., 1967). Training for 24 weeks, two evenings a week, with increasing intensity produced very favourable changes both in mood and muscular endurance (Rechnitzer et al., 1967b). The Frick study (Frick, 1968) involved a group which trained three periods weekly for one

to two months on a bicycle ergometer at a load which produced a heart rate of over 100 beats/min ending with a short period to a level which induced angina or a heart rate of 150 beats/min. The training resulted in a reduction of exercise heart rate and tension-time index, and enhancement of stroke volume. Left ventricular function was improved and the exercise tolerance increased.

Evidently, patients with a healed myocardial infarction can derive great benefit from an increase in physical activity, but type, optimal level and time after infarct to start training have not been well established. Physiological responses are characterized by significant reduction of the resting pulse rate, systolic and diastolic blood pressures both at rest and during comparable levels of energy expenditures and by a significant increase in physical working capacity (Naughton et al., 1966; Hellerstein and Hornstein, 1966). According to Naughton et al., (1969) these patients differed (clinically) following training in that they worked to greater levels with less sensation of discomfort and fatigue. The decrease in pulse rate and systolic blood pressure indicated that the training process was accompanied by a significant reduction in myocardial oxygen consumption. These changes were also reflected in the systolic tension-time index reported by Hellerstein (1966).

Although few studies relating the alternations in blood flow which occur as a result of regular physical activity have been reported, some findings suggest that the trained cardiac patient has a larger resting stroke volume than a comparable sedentary but otherwise healthy individual. Crews and Aldinger (1967) reported that chronic exercise

increased myocardial function. Whitsett and Naughton (1968) reported that cardiac patients who engaged in regular physical activity responded to moderate levels of physical exercise with a significant shortening of the left ventricular ejection time, whereas it was prolonged during test exercise in the sedentary groups of patients. These findings suggest that regular physical activity enhances the strength of myocardial contractility even in the presence of known myocardial disease, although the exact mechanism remains to be explained.

EFFECT OF EXERCISE ON CATECHOLAMINE LEVELS

There is substantial evidence that the catecholamine levels in plasma are increased after severe or prolonged muscular work and exercise. Gray and Beetham (1957) found that plasma levels of noradrenaline increased significantly after severe exercise whereas the plasma adrenaline was more variable. Munro and Robinson (1958) found a rise in norepinephrine levels but not in epinephrine; when the subject ran a quarter mile or more, the plasma epinephrine levels were increased. Vendsalu (1960) found increased levels of catecholamines after exercise. Klensch (1966) found a rise in venous plasma noradrenaline from 0.2 to 0.42 ng/ml. after exercise at 100 watts for ten minutes. The adrenaline level only rose if the exercise was prolonged for a further 10 minutes.

When two normal men performed muscular work at rates of 600 Kg-m/min for 30 minutes, their plasma noradrenaline levels rose by four and six-fold, but the plasma levels of conjugated noradrenaline rose only by about 50% (Haggendal, 1963). Similar effects of exercise have also been shown

on the urinary excretion of catecholamines by Euler (1966) and Banister (1966).

Gazes, Richardson and Woods (1959) found increases in the plasma catecholamines after exercise in patients suffering from angina pectoris, with little or no change in normal subjects. Marked differences in the responses of patients with congestive heart failure and normal subjects have been observed (Chidsey et al., 1962; Braunwald et al., 1963). These workers found that patients with heart disease without congestive failure responded in exactly the same way as normal subjects to moderate exercise (100 ft-lbs/min. at 40 rpm for four to six min). There was a slight rise in plasma noradrenaline level together with an increase in heart rate and oxygen consumption. However, in congestive heart failure the resting noradrenaline level tended to be higher than normal, in some cases markedly so, and after moderate exercise the plasma noradrenaline levels rose considerably. There were no consistent changes in the plasma A levels in any of the groups. Normal subjects were subjected to more severe exercise of 3300 ft-lb/min at 60 rev/min for 6 minutes. This procedure rather more than doubled the plasma noradrenaline level.

Christensen (1971) measured plasma catecholamines in 5 young males and in 2 juvenile diabetics during ketosis as well as after a period of insulin treatment. Plasma catecholamines were twice as high at rest, and the increase during exercise was approximately 8 times higher in the ketolic diabetics compared to controls. Häggendal et al. (1970), measured the noradrenaline levels in arterial blood plasma at rest and during increasing muscular exercise in 5 healthy men and found the levels to be significantly correlated to the oxygen consumption, measured at submaximal and calculated at supramaximal work.

CHAPTER III

METHODS

SUBJECTS AND DATA COLLECTION

This study consisted of two groups of subjects:

Group A: Normal Healthy Males

Five adult, healthy males, ranging in age from 23-39 years and experienced in exercise training, performed at various work loads (900, 1200, 1500, 1800 Kgm/min) and samples of venous blood were taken at rest, 3, 6, 9 minutes of exercise and in recovery at 3 and 6 minutes. Plasma catecholamine secretion during and in recovery from exertion was investigated to determine the optimal time of post exercise blood sampling reflecting circulating exercise levels. During each of these tasks, oxygen uptake ($\dot{V}O_2$) was measured during unloaded pedalling and throughout the 9 minutes of exercise, where this could be completed, and at 3 and 6 minutes after the end of the task. In the case where 9 minutes of work could not be completed (1800 Kgm/min) $\dot{V}O_2$ was measured each minute until exhaustion and in recovery as before.

Group B: Post Myocardial Infarction Patients

Fourteen adult, male post infarct patients were matched as closely as possible on the basis of clinical history, age, height, weight and response to an initial exercise tolerance test. They were subdivided into three sub-groups:

Group I - trained on the Von Döbeln bicycle ergometer by the cyclic method, which consisted of alternate high effort (60% of the maximum work capacity determined by the PWC 190) and low effort (40% of the PWC 190). They exercised as follows: five minutes of unloaded cycling, four minutes of high effort, two minutes of unloaded cycling (which was a rest interval). This was followed by four minutes of low effort and two minutes of unloaded cycling. Each session comprised three high-low effort series. Continuous ECG monitoring of the patients was followed throughout. Total training time was forty-five minutes. This group had five subjects.

Group II - This group performed at a moderate resistance of 50% of maximum work capacity (determined by PWC 190) continuously throughout the exercise period. Each training session comprised a five minute warm-up, thirty minutes of continuous cycling and a five minute recovery with continuous ECG monitoring at all stages of the work. Training took place five days per week. This group had five subjects.

Group III - This group participated in a calisthenics-jogging program three days per week. This comprised thirty minutes of calisthenics, followed by twenty minutes of a run-walk series. On the two days between training sessions, the participants walked briskly for thirty minutes each day. This group had only four subjects.

The average age of the members of Group B was 47 years and they were, on average, 5 months post myocardial infarction. Table I summarizes pertinent physical traits, diagnosed functional classification, diagnosed condition, and initial type of training of each participant of Group B.

The experiment ran for 24 weeks. Exercise tolerance of all 14 men was first established on the bicycle ergometer (3 tests). These sub-maximal tests were run to the limits of the patients' subjective tolerance, angina, a heart rate limit of 170 beat/min. or electrocardiographic changes.

The experiment was conducted with an alternating design. The initial exercise tests were carried out in the first two weeks, after which systematic training began for men in each subgroup of Group B.

After ten weeks of training the continuous effort group switched to cyclic training and the cyclic to continuous. These two groups trained for another ten weeks while the calisthenic-jogging group continued their original type of training. Finally, all three groups trained by the continuous method. Exercise took place in the physiotherapy departments of two hospitals or in the Human Performance laboratory at Simon Fraser University with a physician immediately available with emergency equipment at all training and testing sessions. A dc defibrillator and resuscitation equipment, including oxygen and appropriate drugs were immediately available during all training and testing periods.

TABLE 1

SUBJECT AND GROUP INFORMATION

Subject	Occupation	Age	Height (cm)	Weight (kgm)	New York Heart Association Functional Classification	Diagnosis*	Months Since MI	Training Group	Initial Type of Training
M.B.	Truck Driver	50	172.0	72.3	2	Inferior MI	3	1	Cyclic
L.M.	Stock Broker	38	180.6	86.0	2	Posterior MI	6	1	Cyclic
N.S.	Tug Boat Worker	54	172.0	78.3	2	Inferior MI	6	1	Cyclic
W.S.	Warehouseman	43	179.7	69.6	2	Inferior MI	4	1	Cyclic
M.W.	Caterer	56	174.3	54.4	3	Diagnosed CI	4	1	Cyclic
Mean		48.2	175.7	72.1			4.6		
±SD		±7.56	±4.16	±11.7			±1.30		
I.B.	Prison Guard	45	176.5	84.6	2	Posterior MI	4	2	Continuous
D.B.	Bus Driver	34	184.6	80.3	2	Anterior MI	7	2	Continuous
F.C.	Type Setter	51	172.0	72.5	2	Anterior MI	5	2	Continuous
H.C.	Officeworker	55	169.8	73.5	2	Posterior MI	6	2	Continuous
H.G.	Customs Inves'or	50	178.5	74.3	2	Inferior MI	6	2	Continuous
Mean		47.0	176.3	77.0			5.6		
±SD		±8.09	±5.80	±5.21			±1.14		
J.D.	Officeworker	45	185.3	90.6	3	Anterior MI	3	3	Calisthenics-Jogging
W.F.	Draftsman	50	170.7	71.9	2	Anterior MI	7	3	Calisthenics-Jogging
E.K.	Minister	48	176.0	88.1	2	Inferior MI	6	3	Calisthenics-Jogging
B.Q.	Manager	41	175.0	74.5	2	Inferior MI	7	3	Calisthenics-Jogging
Mean		46.0	176.8	81.3			5.8		
±SD		±3.92	±6.15	±9.44			±1.89		
Total Group Mean		47	176.2	76.5			5.3		
SD		±6.49	±4.96	±9.26			±1.44		

* MI Myocardial Infarction
CI Coronary Insufficiency

EXERCISE TOLERANCE TESTS

Three exercise tolerance tests were carried out on each of the fourteen patients in Group B according to the method of Wyndham (1967). Each test comprised a six minute warm up period (50 revolutions/min; no load), three six minute progressively increasing work loads, with a five minute pause between each load followed by a five minute pedalling recovery period.

As training progressed, the first work load was increased so that a heart rate of 110 ± 10 was always achieved; the following two work rates were also adjusted appropriately upwards as the men became trained--always however, it was endeavoured to keep the heart rate at each work rate near to 110, 130, 150 beat/min. respectively. The ECG was monitored continuously and from the results of three initial tests, work rate was plotted against heart rate and the physical working capacity at 170 beat/min. (PWC 170) was established. These tests were based on the publications of Sjostrand (1947) and Wahlund (1948). The physical working capacity at 190 beat/min (PWC 190) was then established from these graphs.

Every two weeks at each test session a resting and post-exercise (final work rate) sample of venous blood were taken for catecholamine analysis.

COLLECTION OF BLOOD AND CATECHOLAMINE ANALYSIS

In Group A, a 19 gauge needle butterfly catheter was introduced into the right ante-cubital vein of each subject prior to the beginning of work. This catheter was kept patent by heparin in saline solution.

Twenty ml of blood were taken serially, throughout work and recovery for later analysis of plasma catecholamines (norepinephrine and epinephrine). A control blood sample was taken prior to beginning the work task during a period of unloaded pedalling. This (60 rpm-no load) control period served as a better, less variant base line condition for both oxygen uptake and plasma catecholamines than either of respective measures taken at rest.

In Group B, blood from the antecubital vein was collected at rest and at the end of the final work rate during each test session. The latter sample was collected six minutes post exercise. A polypropylene tube containing ethylene diamine tetra-acetic acid (EDTA) was used for collection. The sample was centrifuged at 3000 rpm. for twenty minutes. Plasma was separated from the sedimented red cells and the volume recorded. Equal volumes of plasma were taken for percentage recovery studies and analysis. The required amounts of norepinephrine and epinephrine, generally 10 mg. of each, were then added to the plasma aliquots. Protein was precipitated by the addition of 1/10 volume of 4 N perchloric acid to each plasma specimen. The solution was shaken vigorously for two minutes and then centrifuged at 30,000 x g for twenty-five minutes at 5-10°C. High speed centrifugation was carried out in polypropylene kimble tubes. After centrifugation, the clear supernatant was removed and its volume recorded. At this point the sample was prepared for catecholamine extraction and may either be analysed directly or frozen and stored for later batch analysis.

It is important that the catecholamines be concentrated or purified to remove fluorescent contaminants arising from the blood. In this procedure the method of Anton and Sayre (1962) is used in which alumina selectively absorbs the catecholamines at pH 8.5.

Aluminum Oxide Activation:

1. A mixture of 100 g. Al_2O_3 plus 500 ml of HCl in a 1-litre beaker is covered with a watch glass and heated at 90-100°C for 45 minutes with continuous and rapid stirring. A magnetic stirrer-hot plate combination is used.
2. The beaker is removed from the heater-stirrer and the heavier particles of alumina are allowed to settle for exactly 1½ minutes. The yellow supernatant and finer particles are then discarded.
3. The remaining alumina is then washed twice with fresh 200 ml. portions of 2 N HCl at 70°C for 10 minutes with continuous stirring and heating. The mixture is allowed to settle and the superinatant decanted as in section 2.
4. A final acid wash with 500 ml. of 2 N HCl for 10 minutes at 50°C is done and the mixture treated again as in section 2.
5. After decanting the final HCl wash, the remaining Al_2O_3 is washed with fresh 200 ml portions of glass distilled water 20-25 times, allowing the heavier particles to settle and decanting the supernatant each time as described in section 2. The final pH of the supernatant

discarded should be above 3.4

6. The alumina is transferred to an evaporating dish and placed in an oven for 1 hour at 120°C. The dried alumina is then heated at 200°C for 2 hours.
7. The activated alumina is then placed in an incubator at 37°C for storage.

Catecholamine Extraction Technique:

1. The clear supernatant of protein-free filtrate described above is accurately pipetted (the volume recorded) into a 50-ml beaker containing 300 mg of activated alumina, 200 mg of Na_2EDTA and 10 mg of sodium metabisulphite. The total volume is then made up to approximately 25 ml with 0.1 N perchloric acid. Under constant rapid stirring with a glass rod attached to a stirring motor, the mixture is brought to and maintained at pH 8.5 by the dropwise addition of sodium hydroxide. This mixture is allowed to react for 4 minutes under constant pH monitoring and rapid stirring.
2. After the stirring has stopped, the Al_2O_3 is allowed to settle for 1½ minutes and the superinatant is aspirated off and discarded.
3. The precipitated Al_2O_3 is washed into a plastic tube with approximately 10 ml distilled water. The tube is stoppered, shaken to suspend the alumina, centrifuged for 1 minute in a clinical centrifuge and the supernatant aspirated off. This wash is repeated a minimum of four times.

4. After the fourth water wash, 4.0 ml of 0.IN perchloric acid is added to the alumina and the tube tightly capped. The catecholamines are eluted by vigorously shaking the tube for 15 minutes in a mechanical shaker.
5. The mixture is then centrifuged in a clinical centrifuge and the supernatant removed and placed in a polypropylene tube. This supernatant is then centrifuged at 30,000 x g for 10 minutes. The clear supernatant is transferred with a Pasteur pipette to a graduated conical centrifuge tube and the volume is recorded. This final solution contains the catecholamines and may be directly assayed or frozen.

The catecholamines were assayed in an Aminco-Bowman spectrophotofluorometer according to the procedure of Griffiths and Leung (1970).

After oxidation with iodine and tautomerization in alkali to form the fluorescent trihydroxy-indole derivatives, determinations can be made (under standard light and temperature conditions) of norepinephrine (pH 6.5) at excitation and emission wavelengths of 380 nm and 480 nm respectively and of adrenaline (pH 4.0) at excitation and emission wavelengths of 425 nm and 500 nm respectively. Because the emission wavelengths of norepinephrine and epinephrine are so close to each other (480 nm and 500 nm respectively), quantities of both catecholamines occur at pH 6.5 and pH 4.0.

Therefore, it is necessary to differentiate them by using the following simultaneous equations in the calculations:

Calculations:

1. $(S\ NE_{6.5})\ x + (SE_{6.5})y = \Delta Rf_{6.5}$
2. $(S\ NE_{4.0})\ x + (S\ E_{4,0})y = \Delta Rf_{4.0}$

where:

x = ngms NE in reaction mixture

y = ngms E in reaction mixture

$\Delta Rf_{6.5}$ = sample Fl - sample blank at pH 6.5

$\Delta Rf_{4.0}$ = sample Fl - sample blank at pH 4.0

$SNE_{6.5}$ = relative Fl units/ngm of NE standard at pH 6.5

$S\ NE_{4.0}$ = relative Fl units/ngm of NE standard at pH 4.0

$S\ E_{6.5}$ = relative Fl units/ngm of E standard at pH 6.5

$S\ E_{4.0}$ = relative Fl units/ngm of E standard at pH 4.0

Concentration of catecholamine in plasma - ngms/ml

$$\text{Concentration in plasma} = x \text{ or } y \frac{(D)}{(E)} \frac{(B)}{(C)} \frac{(1)}{(A)}$$

where

x or y as above concentration of NE or E in ng

A = volume plasma - ml

B = volume of plasma + perchloric acid - ml

C = Volume of protein free filtrate for extraction - ml

D = volume of acid recovered from the alumina - ml

E = aliquot of acid extract assayed - ml

Instrumentation:

An Aminco-Bowman spectrophotofluorometer (SPF) supplied by American Instrument Co. Inc., equipped with a xenon lamp with an off-axis ellipsoidal condensing system was used.

During the assay procedure, the off-axis ellipsoidal system must be reset to an arbitrary relative intensity by use of the entrance slits or focussing attachments. A quinine sulphate solution with a concentration of 1 microgram per cc. is used; set at 350 nm, excitation and 450 nm, emission wavelengths to monitor the SPF.

CHAPTER IV

RESULTS

CIRCULATING PLASMA CATECHOLAMINE ELEVATION AND DECLINE DURING EXERCISE AND RECOVERY IN NORMAL HEALTHY SUBJECTS

Plasma norephinephrine (NE) and epinephrine (E) during unloaded bicycling and exercise are shown in Table II. They were measured in a group of five healthy men at intervals throughout work at four levels of intensity (900, 1200, 1500, 1800 Kg-m/min at 100 rpm). Each exercise lasted nine minutes or until exhaustion.

Throughout each work rate, a progressive increase in the elevation of the circulating catecholamines may be observed and the highest mean values reached by both NE and E occurred just prior to exhaustion.

The progressive increase in the elevation of the circulating catecholamines during exertion was followed by a decline in their levels during recovery but they remain elevated up to six minutes post exercise. These results provide supportive evidence to justify the collection of the post exercise plasma samples from cardiac patients between the third and the sixth minute post exercise, in order to characterize cardiovascular stress.

In healthy subjects, although six minute post exercise circulating plasma catecholamines levels are not as high as immediate post exercise

TABLE II

PLASMA NOREPINEPHRINE AND EPINEPHRINE IN VENOUS BLOOD DURING THE COURSE OF WORK AT DIFFERENT RATES IN 5 MALE SUBJECTS* (MEAN ±SE)

TIME	900 kpm/min		1200 kpm/min		1500 kpm/min			1800 kpm/min	
	NE ng/ml	E ng/ml	NE ng/ml	E ng/ml	NE ng/ml	E ng/ml	TIME	NE ng/ml	E ng/ml
WARM-UP	0.24 ±.04	0.05 ±.01	0.20 ±.03	0.06 ±.01	0.19 ±.02	0.07 ±.01	W-up	0.21 ±.03	0.09 ±.03
E_3	0.32 ±.03	0.08 ±.01	0.61 ±.06	0.10 ±.01	0.69 ±.07	0.16 ±.02	E_1	0.68 ±.02	0.23 ±.04
E_6	0.44 ±.03	0.10 ±.01	0.79 ±.10	0.13 ±.01	1.01 ±.22	0.23 ±.03	E_2	0.89 ±.06	0.38 ±.02
E_9	0.70 ±.08	0.13 ±.02	1.01 ±.07	0.15 ±.02	1.39 ±.09	0.33 ±.05	E_3	2.08 ±.16	0.47 ±.03
R_3	0.44 ±.08	0.08 ±.02	0.60 ±.04	0.10 ±.01	0.84 ±.13	0.22 ±.05	R_3	1.81 ±.07	0.37 ±.05
R_6	0.19 ±.03	0.07 ±.01	0.24 ±.03	0.07 ±.02	0.36 ±.03	0.11 ±.01	R_6	0.54 ±.08	0.16 ±.03

* 1500 kpm/min, n = 4 for E_6 and E_9

n = 4 for R_6

1800 kpm/min, n = 3 for E_2

n = 4 for E_3

values, it is considered that they reflect the cardiovascular and anxiety stress of exercise adequately. The time course of NE and E accumulation (means of five subjects) in plasma during the course of exercise at increasing work rates (900-1800 Kg-m/min) are illustrated in Figures 8 and 9. The mean increase in circulating NE and E from rest to peak exercise levels was in all cases significant ($p<.01$).

RESPIRATORY GAS EXCHANGE

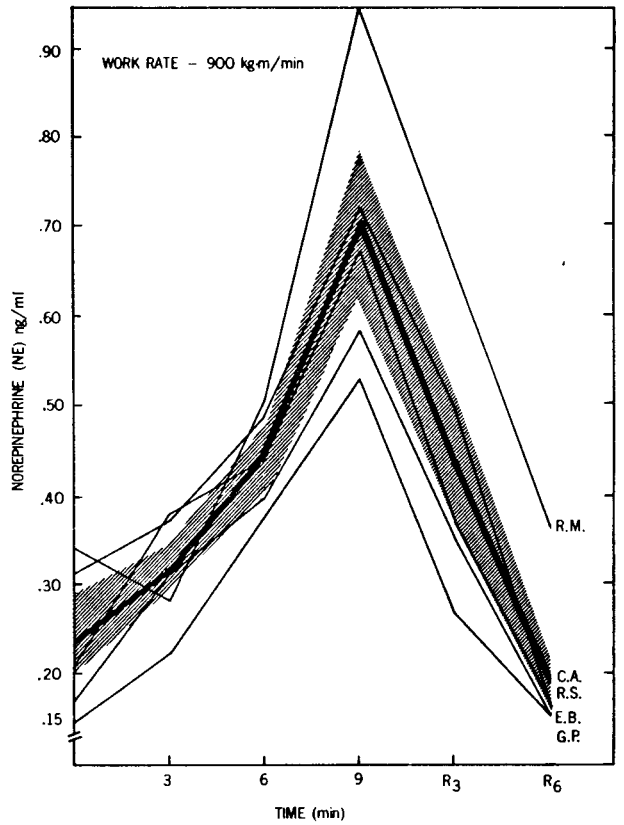
Table III shows the mean respiratory gas exchange for the same five subjects during each work rate. It may be seen that circulating norepinephrine and epinephrine concentrations increased and showed a correspondence both with the degree of intensity of exercise and the oxygen uptake.

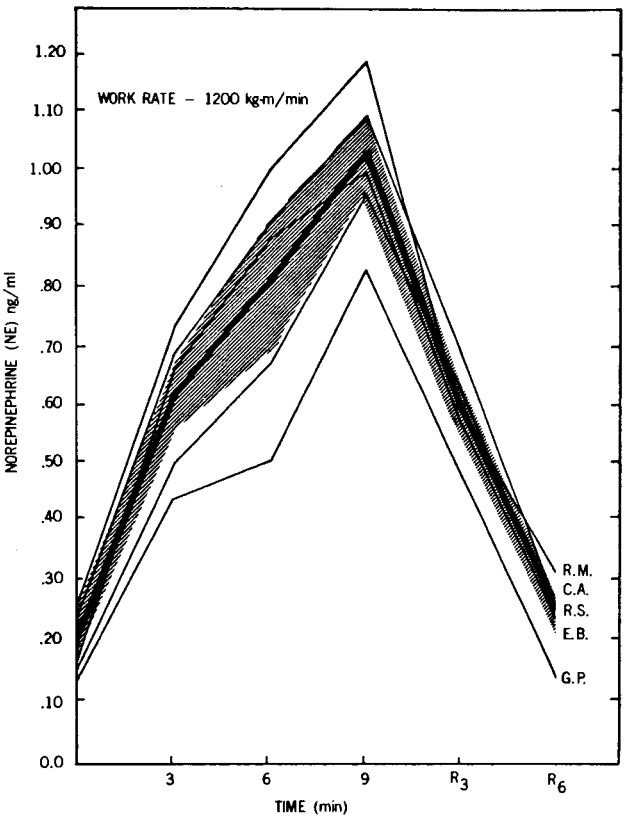
The oxygen consumption ($\dot{V}O_2$) for the five subjects during each of the work rates is shown in Figure 10. The oxygen consumption closely parallels the rise in circulating catecholamines indicating the degree of stress experienced by these subjects with increasing work levels. Similarly, the work levels of cardiac patients probably represents as big a stress to them as in the case of the normal subjects and similar time sequences are applicable. Since rebreathing measurements were taken during the course of exercise by the cardiac patients for the assessment of cardiac output, it was not possible to sample for circulating catecholamines at times similar to the normals.

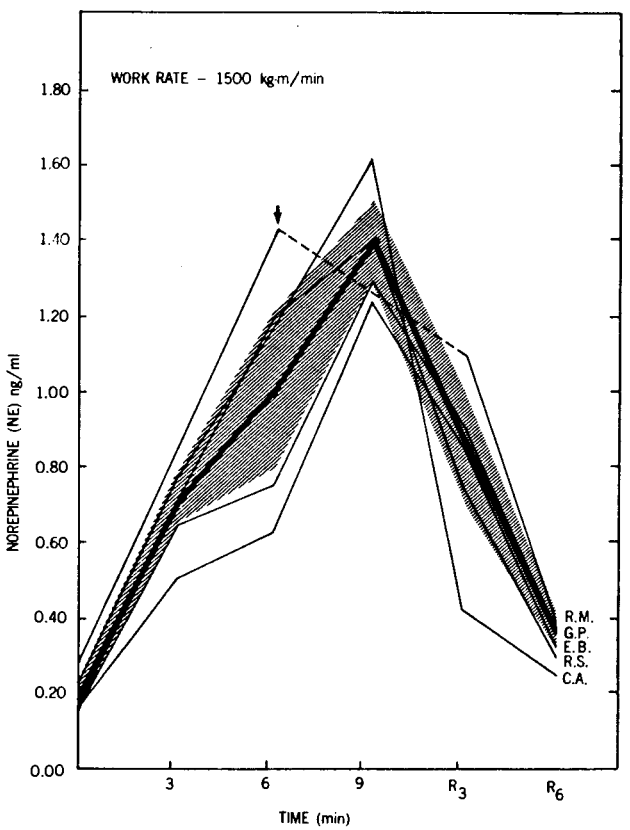
Figure 8. Time course of norepinephrine (NE) accumulation in plasma during exercise at 900, 1200, 1500 and 1800 Kg-m/min by five, healthy male subjects.

Arrows indicate points of cessation of exercise and dotted lines are drawn from the points of cessation of exercise to the third minute of recovery sample.

Thick line and shaded area represent the group mean ± S.E. Absence of these latter lines indicates the lack of adequate group data necessary for their calculation.


WORK RATE – 900 kg·m/min
NOREPINEPHRINE (NE) ng/ml
.90
.80
.70
.60
.50
.40
.30
.20
.15
R.M.
C.A.
R.S.
E.B.
G.P.
3
6
9
R3
R6
TIME (min)


WORK RATE – 1200 kg·m/min
NOREPINEPHRINE (NE) ng/ml
1.20
1.10
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
0.0
R.M.
C.A.
R.S.
E.B.
G.P.
3
6
9
R3
R6
TIME (min)


WORK RATE – 1500 kg·m/min
NOREPINEPHRINE (NE) ng/ml
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
R.M.
G.P.
E.B.
R.S.
C.A.
3
6
9
R3
R6
TIME (min)

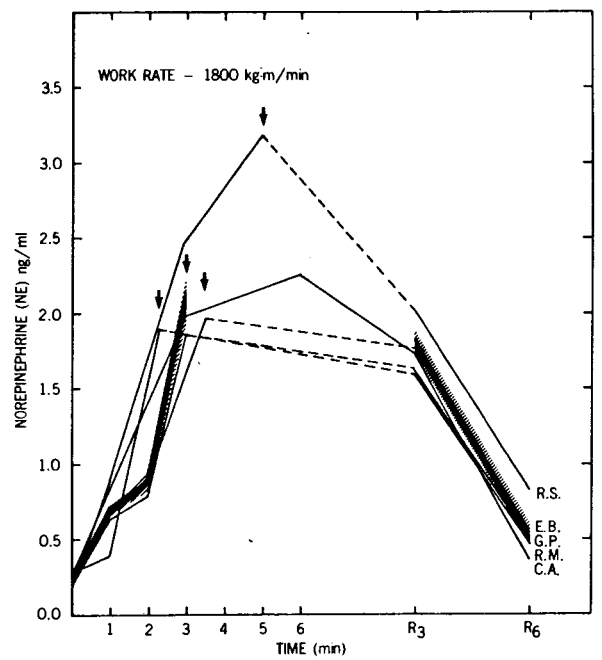
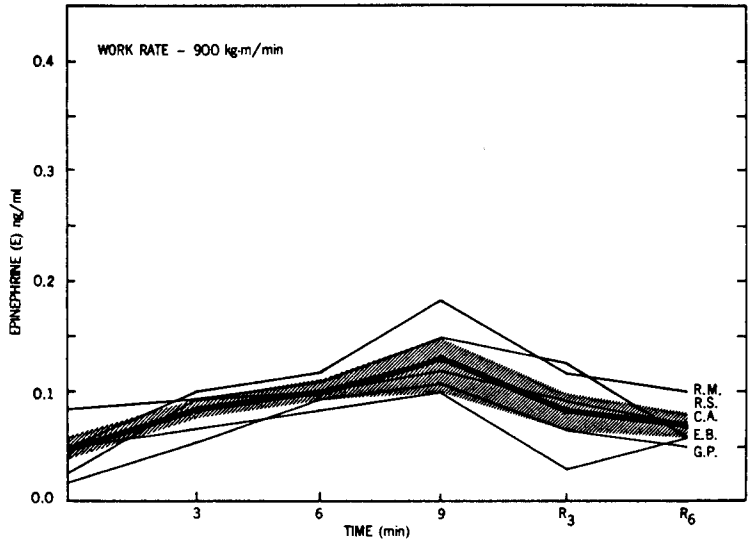
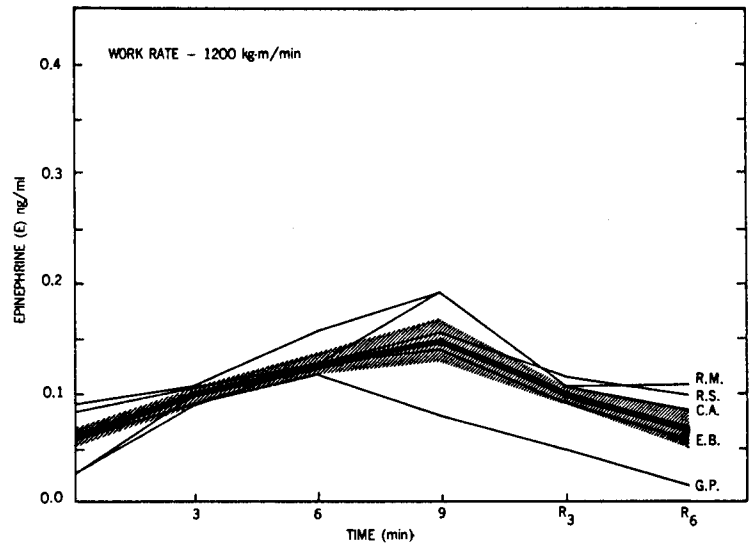

WORK RATE – 1800 kg·m/min
NOREPINEPHRINE (NE) ng/ml
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
R.S.
E.B.
G.P.
R.M.
C.A.
1
2
3
4
5
6
R3
R6
TIME (min)

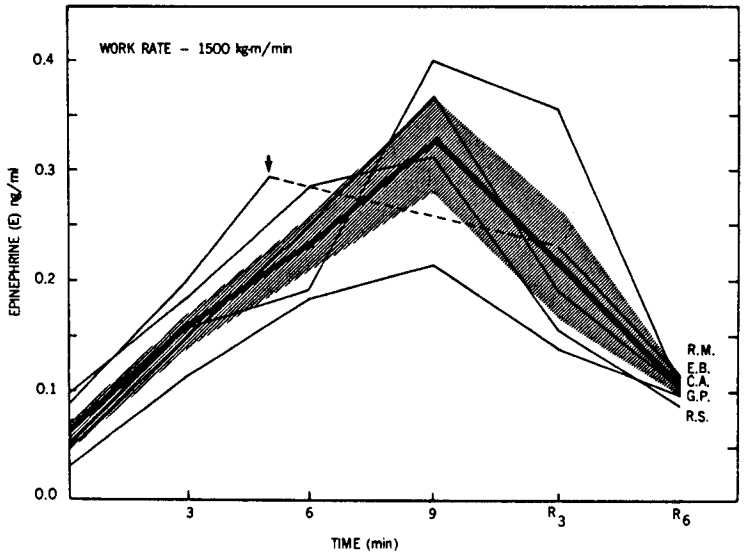
Figure 9. Time course of epinephrine (E) accumulation in plasma during exercise at 900, 1200, 1500 and 1800 Kg-m/min by five, healthy male subjects.

Arrows indicate points of cessation of exercise and dotted lines are drawn from the points of cessation of exercise to the third minute of recovery sample.

Thick line and shaded area represent the group mean ± S.E. Absence of these latter lines indicates the lack of adequate group data necessary for their calculation.


WORK RATE – 900 kg-m/min
EPINEPHRINE (E) ng/ml
0.0
0.1
0.2
0.3
0.4
3
6
9
R3
R6
TIME (min)
R.M.
R.S.
C.A.
E.B.
G.P.


WORK RATE – 1200 kg-m/min
EPINEPHRINE (E) ng/ml
0.0
0.1
0.2
0.3
0.4
3
6
9
R3
R6
TIME (min)
R.M.
R.S.
C.A.
E.B.
G.P.


WORK RATE – 1500 kg-m/min
EPINEPHRINE (E) ng/ml
0.0
0.1
0.2
0.3
0.4
3
6
9
R3
R6
TIME (min)
R.M.
E.B.
C.A.
G.P.
R.S.

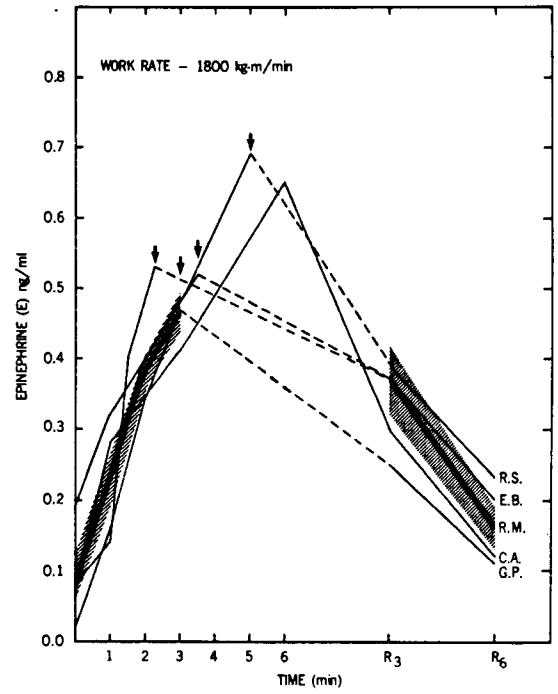

WORK RATE – 1800 kg-m/min
EPINEPHRINE (E) ng/ml
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
1
2
3
4
5
6
R3
R6
TIME (min)
R.S.
E.B.
R.M.
C.A.
G.P.

TABLE III

RESPIRATORY GAS EXCHANGE DURING THE COURSE OF WORK AT DIFFERENT RATES IN 5 MALE SUBJECTS (MEAN ± S.E.)

TIME	900 kpm/min			1200 kpm/min			1500 kpm/min			TIME	1800 kpm/min		
	$\dot{V}O_2$	$\dot{V}CO_2$	R	$\dot{V}O_2$	$\dot{V}CO_2$	R	$\dot{V}O_2$	$\dot{V}CO_2$	R		$\dot{V}O_2$	$\dot{V}CO_2$	R
WARM-UP	0.57 ±.03	0.42 ±.04	0.74 ±.05	0.52 ±.05	0.41 ±.04	0.78 ±.03	0.62 ±.04	0.51 ±.04	0.83 ±.05	WARM-UP	0.64 ±.08	0.57 ±.10	0.88 ±.06
E_3	2.46 ±.08	2.22 ±.09	0.94 ±.03	2.80 ±.08	2.86 ±.04	1.02 ±.03	3.47 ±.20	3.75 ±.14	1.09 ±.05	E_1	2.04 ±.15	1.98 ±.17	0.98 ±.07
E_6	2.46 ±.13	2.30 ±.12	0.93 ±.02	3.16 ±.09	3.07 ±.10	0.97 ±.01	4.01 ±.09	4.28 ±.14	1.07 ±.05	E_3	3.41 ±.43	3.98 ±.42	1.18 ±.06
E_9	2.59 ±.16	2.40 ±.17	0.94 ±.03	3.32 ±.13	3.13 ±.10	0.94 ±.01	4.06 ±.13	4.13 ±.19	1.02 ±.05	E_4	3.92 ±.27	4.37 ±.07	1.13 ±.08
R_3	0.76 ±.21	0.81 ±.17	1.16 ±.10	0.76 ±.05	0.87 ±.08	1.15 ±.04	0.97 ±.05	1.17 ±.09	1.20 ±.05	E_5	4.49 ±.06	4.49 ±.15	1.00 ±.01
R_6	0.56 ±.05	0.52 ±.10	1.11 ±.13	0.59 ±.03	0.56 ±.02	0.95 ±.02	0.85 ±.07	0.88 ±.06	1.03 ±.04	R_3	1.05 ±.13	1.28 ±.23	1.21 ±.07
										R_6	0.82 ±.01	0.89 ±.10	1.09 ±.13

900 kpm/min, n = 3 for R_3 and R_6

1200 kpm/min, n = 4 for R_3 and R_6

1500 kpm/min, n = 4 for E_6 and E_9

1800 kpm/min, n = 4 for E_3

n = 4 for E_4

n = 2 for E_5

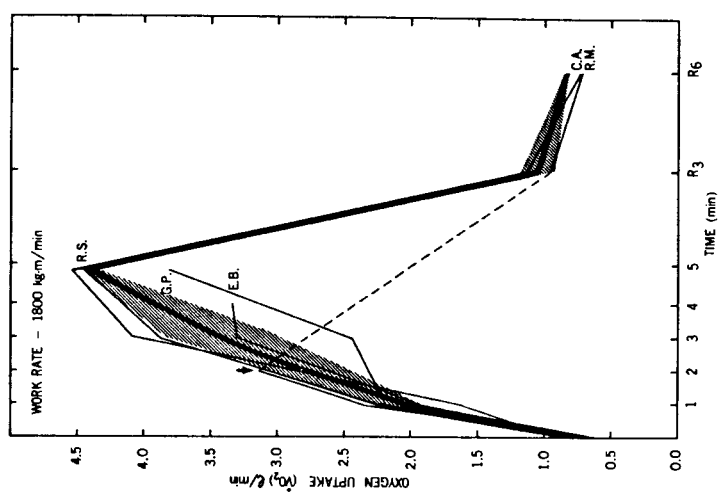
n = 2 for R_3 and R_6

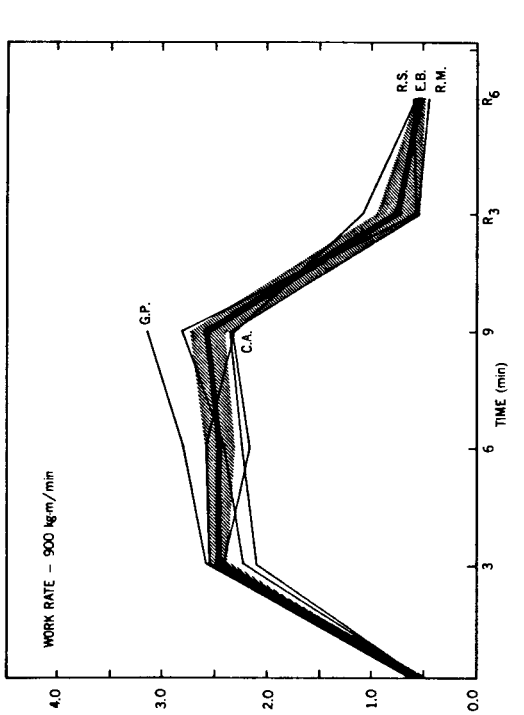
Figure 10. Changes in oxygen intake ($\dot{V}O_2$) during exercise at 900, 1200, 1500 and 1800 Kg-m/min by five, healthy male subjects.

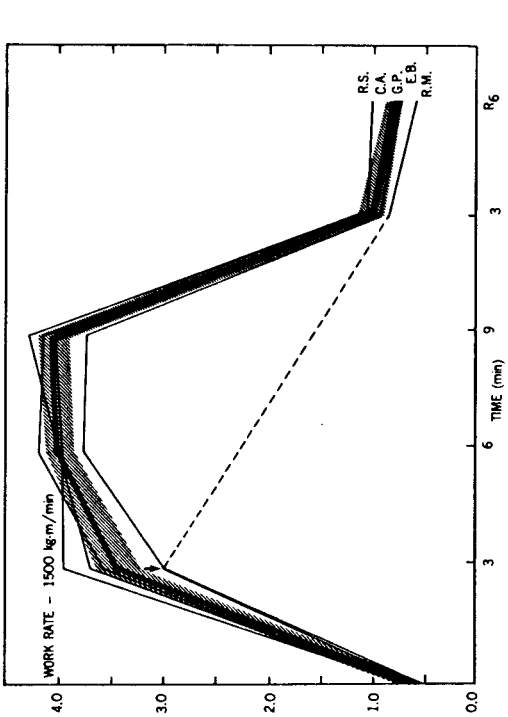
Arrows indicate points of cessation of exercise and dotted lines are drawn from the points of cessation of exercise to the third minute of recovery sample.

Thin line and shaded area represent the group mean ± S.E.


WORK RATE – 1200 kg·m/min
OXYGEN UPTAKE ($\dot{V}O_2$) ℓ/min
TIME (min)
R.S.
G.P.
C.A.
R.M.
E.B.
R_3
R_6


WORK RATE – 1800 kg·m/min
OXYGEN UPTAKE ($\dot{V}O_2$) ℓ/min
TIME (min)
R.S.
G.P.
E.B.
C.A.
R.M.
R_3
R_6


WORK RATE – 900 kg·m/min
OXYGEN UPTAKE ($\dot{V}O_2$) ℓ/min
TIME (min)
G.P.
C.A.
R.S.
E.B.
R.M.
R_3
R_6


WORK RATE – 1500 kg·m/min
OXYGEN UPTAKE ($\dot{V}O_2$) ℓ/min
TIME (min)
R.S.
C.A.
G.P.
E.B.
R.M.
R_3
R_6

CIRCULATING PLASMA CATECHOLAMINE ELEVATION AND DECLINE DURING EXERCISE AND RECOVERY IN THREE GROUPS OF POST MYOCARDIAL INFARCT (M.I.) PATIENTS

(Computer program for a split plot two factor, design analysis of variance (Appendix I)).

F ratios and significant differences in all the variables studied are shown in Table IV. This table indicates significant positive changes with training in all the groups in terms of decreased plasma epinephrine at rest and decreased plasma norepinephrine both at rest and during exercise. There was an increased oxygen consumption concomitant with an increased work capacity and an interraction between training groups in terms of heart rate.

GROUP I (WORK RATE, CATECHOLAMINES, HEART RATE AND OXYGEN CONSUMPTION)

The mean values for the work rate, plasma norepinephrine and epinephrine, heart rate and oxygen consumption for Group I at rest and during exercise are presented in Table V. This group trained initially on a von Döbeln bicycle ergometer alternately at high effort (60%) of the maximum work capacity determined by the PWC_{190}, and low effort (40%) of the PWC_{190}.

Individual measurements, during twenty-four weeks of training for each of the test variables are shown in Figures 11 to 14. The mean values at each test for the parameters suggest the general group trend of progress.

TABLE IV

RESULTS OF THE SPLIT PLOT TWO FACTOR, DESIGN ANALYSIS OF VARIANCE, WHERE FACTOR A IS BETWEEN GROUPS, FACTOR B IS WITHIN GROUPS AND FACTOR AB IS THE INTERACTION. SIGNIFICANT DIFFERENCES BETWEEN MEANS WERE DETERMINED BY THE SCHEFFE METHOD

Parameter	Factor	F Ratio	Level of Significance	Scheffe's Factor for Significant Differences Between Means)	Significant Differences Between Means
Epinephrine (E) (Rest)	A	0.50	None		
	B	1.82	*	.0115	i,ii,iii,iv,v, vi,vii,xi,xii.
	AB	0.87	None		
Epinephrine (E) (Exercise)	A	1.31	None		
	B	1.07	None		
	AB	0.77	None		
Norepinephrine (NE) (Rest)	A	2.18	None		
	B	2.35	***	.118	ii,iv,xii.
	AB	0.79	None		
Norepinephrine (NE)(Exercise)	A	1.61	None		
	B	2.31	**	.141	iv,vii,xi.
	AB	0.72	None		
Heart Rate (H.R.)	A	1.63	None		
	B	0.69	None		
	AB	1.73	**	10.18	Interaction between Groups I, II and III.
Oxygen Consumption ($\dot{V}O_2$)	A	0.31	None		
	B	4.62	***	.276	iv,v,vii,ix.
	AB	1.11	None		

a. * Significance at .10
A df 2,11 F = 2.86
B df 12,132 F = 1.60
AB df 24,132 F = 1.45

** Significance at .05
A df 2,11 F = 3.89
B df 12,132 F = 1.83
AB df 24,132 F = 1.61

*** Significance at .01
A df 2,11 F = .01
B df 12,132 F = 2.34
AB df 24,132 F = 1.95

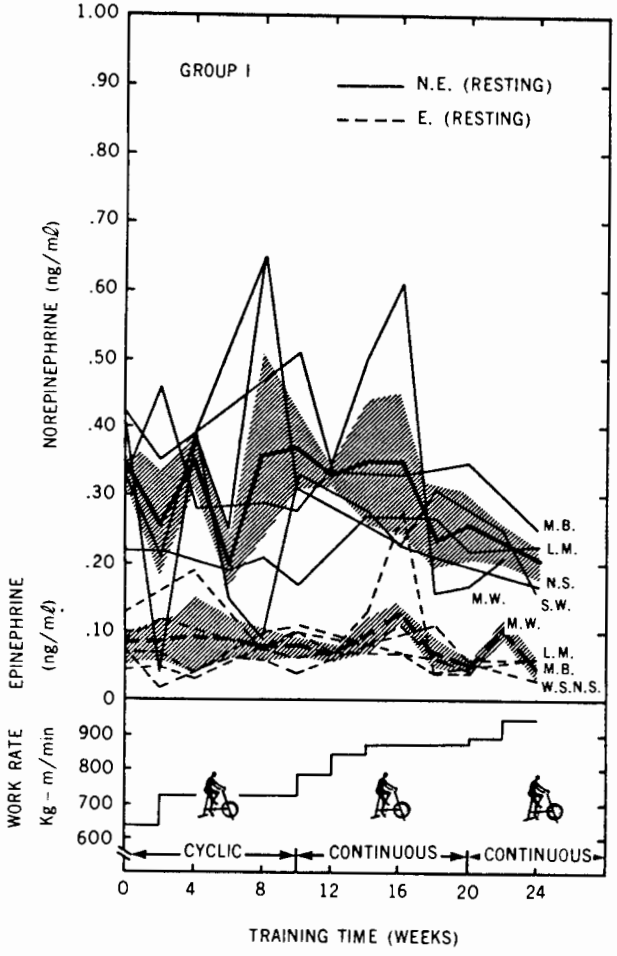
b. i. Significant difference between initial and 10 weeks.
ii. Significant difference between initial and 18 weeks.
iii. Significant difference between initial and 20 weeks.
iv. Significant difference between initial and 24 weeks.
v. Significant difference between 2 and 14 weeks.
vi. Significant difference between 2 and 18 weeks.
vii. Significant difference between 2 and 24 weeks.
viii. Significant difference between 4 and 14 weeks.
ix. Significant difference between 4 and 18 weeks.
x. Significant difference between 10 and 20 weeks.
xi. Significant difference between 10 and 24 weeks.
xii. Significant difference between 20 and 24 weeks.

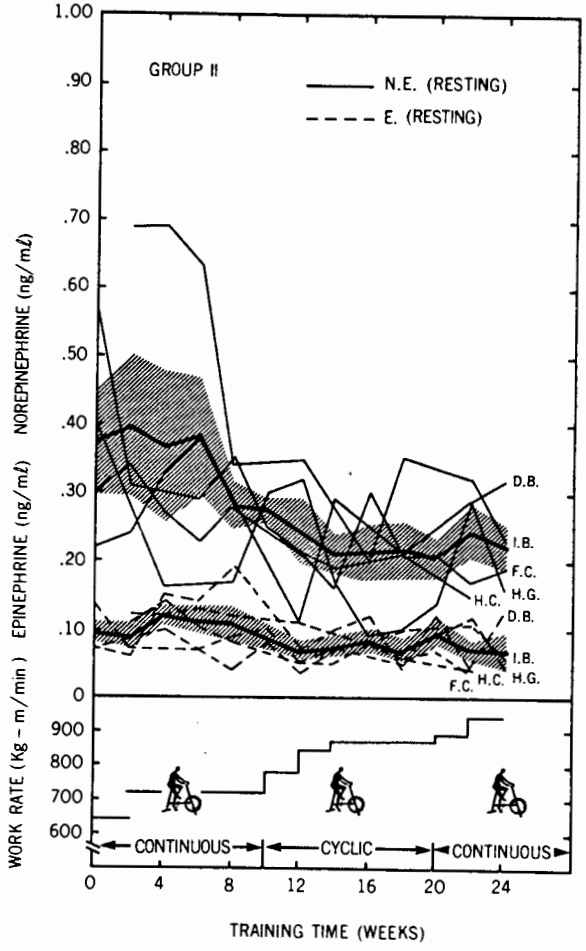
TABLE V

WORK RATE (W.R.), PLASMA NOREPINEPHRINE (NE), PLASMA EPINEPHRINE (E), HEART RATE (H.R.) AND OXYGEN CONSUMPTION ($\dot{V}O_2$) OF 5 MALE SUBJECTS IN GROUP I DURING A 24 WEEK TRAINING PERIOD (MEAN ± S.E.)

Time (Week)	W.R. (Kg-m/min)	NE (ng/ml)		E (ng/ml)		H.R. beat/min		$\dot{V}O_2$ L/min
		Rest	Exercise	Rest	Exercise	Warm Up	Exercise	
0	639 ± 77	.34 ± .04	.64 ± .10	.08 ± .02	.18 ± .03	85 ± 5.2	152 ± 9.6	2.15 ± .43
2	720 ± 63	.26 ± .08	.47 ± .15	.08 ± .03	.15 ± .06	74 ± 2.1	160 ± 4.8	2.17 ± .14
4	720 ± 59	.35 ± .04	.49 ± .08	.09 ± .06	.11 ± .01	75 ± 1.9	152 ± 7.3	2.02 ± .15
6	620 ± 63	.20 ± .04	.45 ± .11	.09 ± .03	.16 ± .05	83 ± 2.2	156 ± 5.8	2.00 ± .12
8	720 ± 63	.31 ± .14	.57 ± .11	.08 ± .02	.21 ± .05	75 ± 1.3	151 ± 5.7	2.11 ± .14
10	780 ± 34	.32 ± .06	.66 ± .15	.08 ± .02	.16 ± .05	77 ± 2.2	151 ± 6.0	2.05 ± .14
12	840 ± 41	.34 ± .01	.47 ± .07	.07 ± .01	.15 ± .04	80 ± 2.2	151 ± 6.0	2.04 ± .08
14	870 ± 63	.35 ± .09	.45 ± .04	.10 ± .02	.16 ± .04	73 ± 1.0	156 ± 2.4	2.32 ± .15
16	870 ± 63	.35 ± .10	.57 ± .02	.13 ± .06	.19 ± .05	81 ± 6.6	156 ± 1.8	2.27 ± .11
18	870 ± 63	.24 ± .04	.47 ± .08	.07 ± .02	.16 ± .03	77 ± 2.2	139 ± 8.7	2.17 ± .13
20	886 ± 55	.26 ± .04	.44 ± .06	.05 ± .01	.15 ± .05	74 ± 1.7	145 ± 3.2	2.17 ± .08
22	945 ± 63	.23 ± .03	.42 ± .07	.10 ± .01	.15 ± .09	77 ± 1.8	155 ± 3.1	2.15 ± .04
24	945 ± 63	.20 ± .03	.39 ± .03	.04 ± .01	.09 ± .01	84 ± .45	155 ± 4.4	2.14 ± .10

Figure 11. Changes in plasma norepinephrine (NE) and plasma epinephrine (E) (Rest) during twenty-four weeks of training in Groups I, II and III. (Thick line and shaded area group mean ± S.E.).


GROUP I
N.E. (RESTING)
E. (RESTING)
NOREPINEPHRINE (ng/mℓ)
EPINEPHRINE (ng/mℓ)
WORK RATE Kg - m/min
M.B.
L.M.
N.S.
S.W.
M.W.
M.W.
L.M.
M.B.
W.S.N.S.
CYCLIC
CONTINUOUS
CONTINUOUS
TRAINING TIME (WEEKS)


GROUP II
N.E. (RESTING)
E. (RESTING)
NOREPINEPHRINE (ng/mℓ)
EPINEPHRINE (ng/mℓ)
WORK RATE (Kg - m/min)
D.B.
I.B.
F.C.
H.G.
H.C.
D.B.
I.B.
F.C.
H.C.
H.G.
CONTINUOUS
CYCLIC
CONTINUOUS
TRAINING TIME (WEEKS)

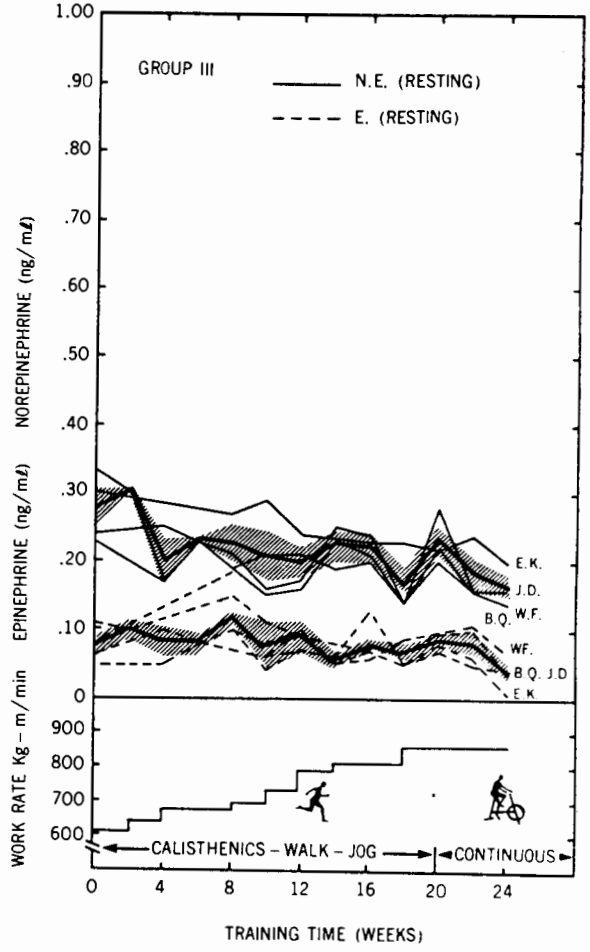
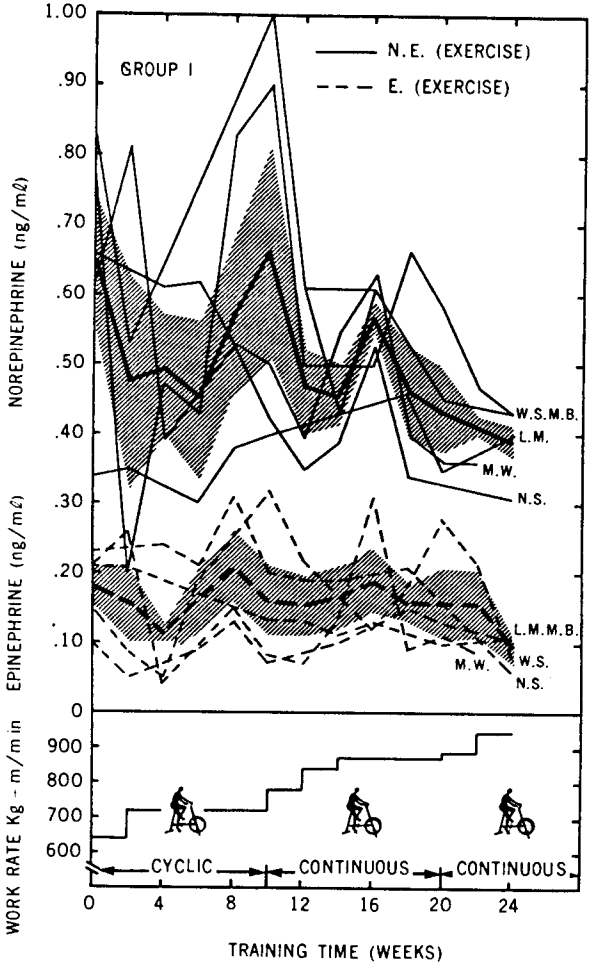
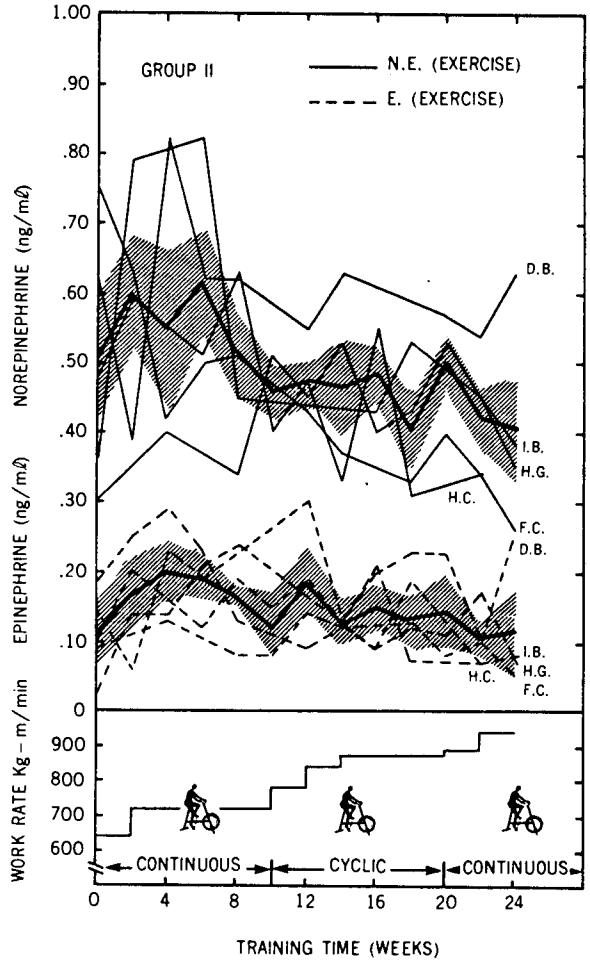

GROUP III
N.E. (RESTING)
E. (RESTING)
NOREPINEPHRINE (ng/mℓ)
EPINEPHRINE (ng/mℓ)
WORK RATE Kg - m/min
E.K.
J.D.
B.Q.
W.F.
WF.
B.Q.
J.D.
E.K.
CALISTHENICS - WALK - JOG
CONTINUOUS
TRAINING TIME (WEEKS)

Figure 12. Changes in plasma norepinephrine (NE) and plasma epinephrine (E) (Exercise) during twenty-four weeks of training in Groups I, II and III. (Thick line and shaded area group mean ± S.E.).


GROUP I
N.E. (EXERCISE)
E. (EXERCISE)
NOREPINEPHRINE (ng/mℓ)
EPINEPHRINE (ng/mℓ)
W.S.M.B.
L.M.
M.W.
N.S.
L.M.M.B.
W.S.
M.W.
N.S.
WORK RATE Kg - m/min
CYCLIC
CONTINUOUS
CONTINUOUS
TRAINING TIME (WEEKS)


GROUP II
N.E. (EXERCISE)
E. (EXERCISE)
NOREPINEPHRINE (ng/mℓ)
EPINEPHRINE (ng/mℓ)
D.B.
I.B.
H.G.
H.C.
F.C.
D.B.
I.B.
H.G.
H.C.
F.C.
WORK RATE Kg - m/min
CONTINUOUS
CYCLIC
CONTINUOUS
TRAINING TIME (WEEKS)

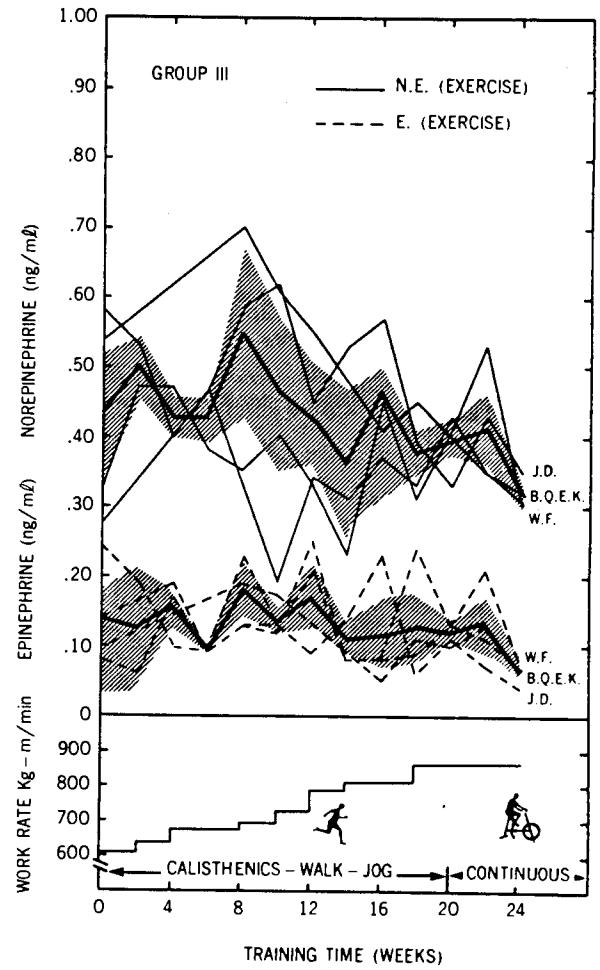
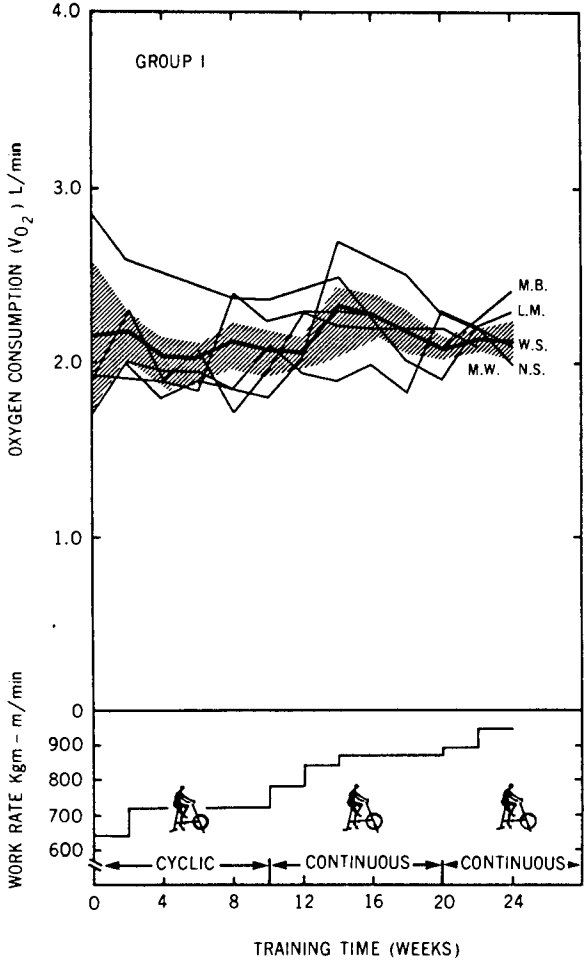
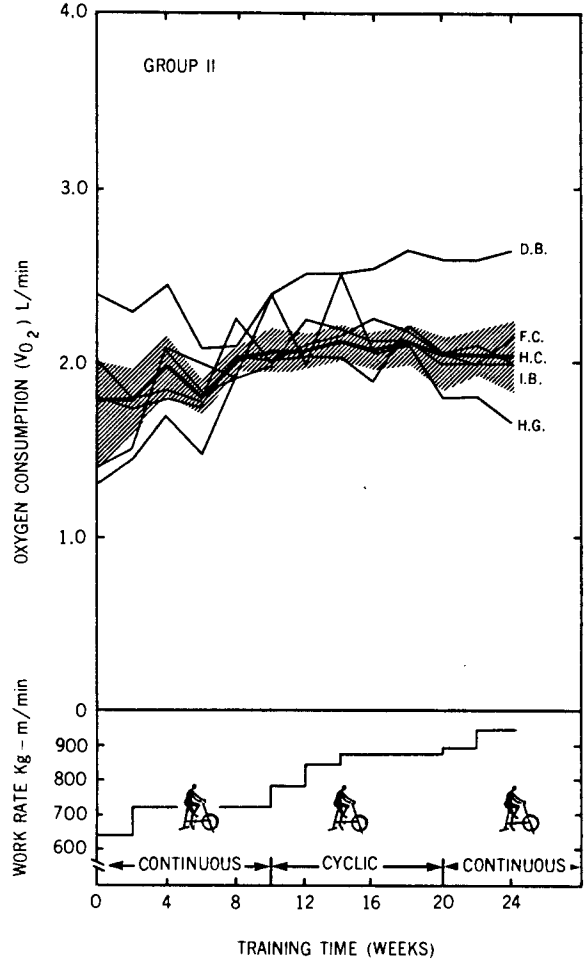

GROUP III
N.E. (EXERCISE)
E. (EXERCISE)
NOREPINEPHRINE (ng/mℓ)
EPINEPHRINE (ng/mℓ)
J.D.
B.Q.E.K.
W.F.
W.F.
B.Q.E.K.
J.D.
WORK RATE Kg - m/min
CALISTHENICS - WALK - JOG
CONTINUOUS
TRAINING TIME (WEEKS)

Figure 13. Changes in oxygen consumption ($\dot{V}O_2$) during twenty-four weeks of training in Groups I, II and III. (Thick line and shaded area group mean ± S.E.).


GROUP I
OXYGEN CONSUMPTION (V_{O_2}) L/min
4.0
3.0
2.0
1.0
0
M.B.
L.M.
W.S.
M.W.
N.S.
WORK RATE Kgm - m/min
900
800
700
600
CYCLIC
CONTINUOUS
CONTINUOUS
0 4 8 12 16 20 24
TRAINING TIME (WEEKS)


GROUP II
OXYGEN CONSUMPTION (V_{O_2}) L/min
4.0
3.0
2.0
1.0
0
D.B.
F.C.
H.C.
I.B.
H.G.
WORK RATE Kg - m/min
900
800
700
600
CONTINUOUS
CYCLIC
CONTINUOUS
0 4 8 12 16 20 24
TRAINING TIME (WEEKS)

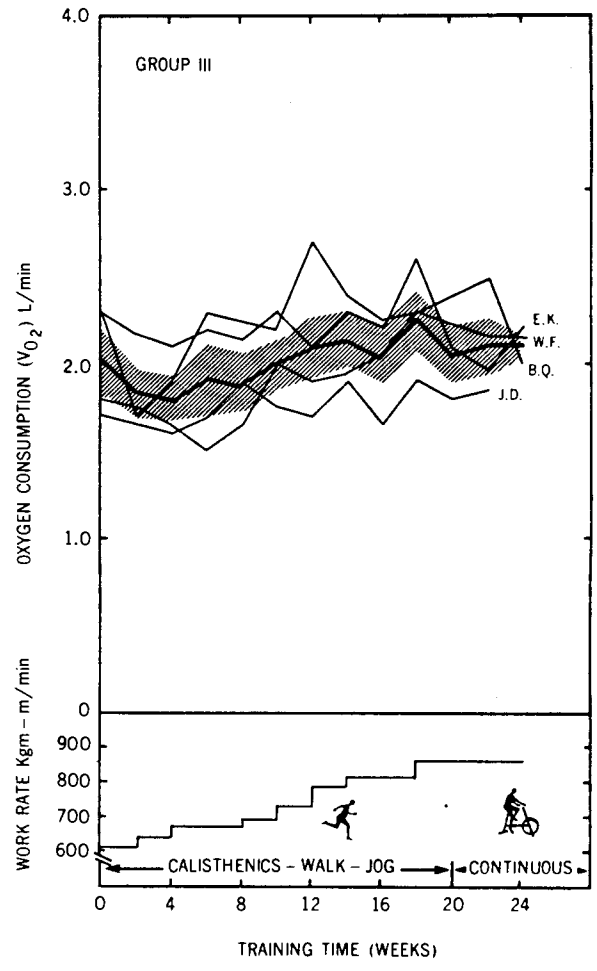
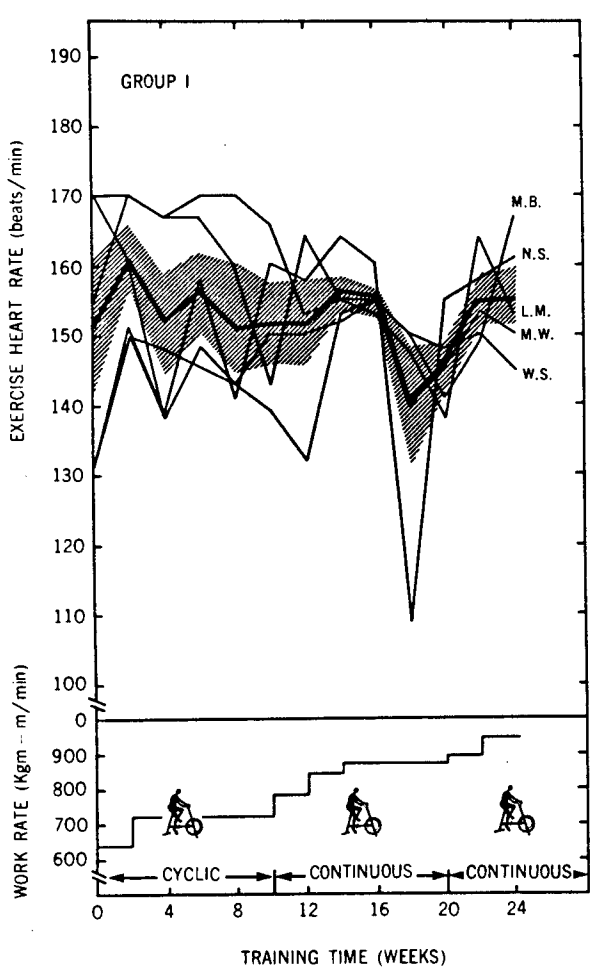
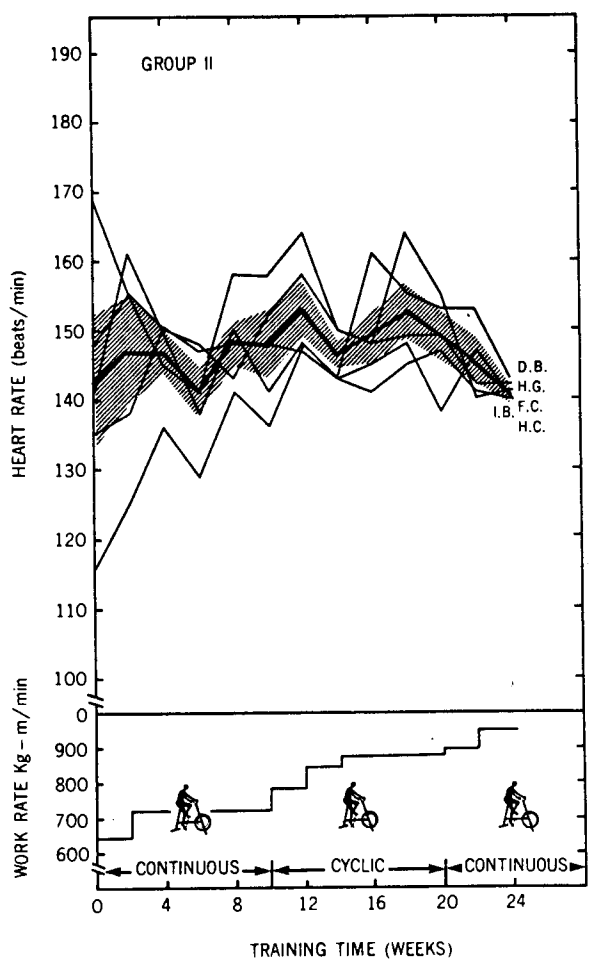
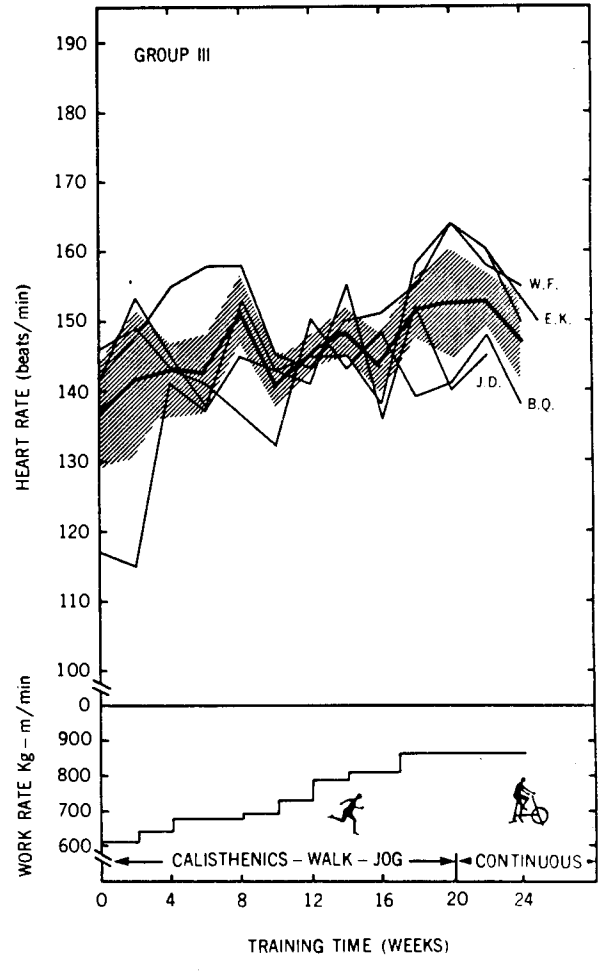

GROUP III
OXYGEN CONSUMPTION (V_{O_2}) L/min
4.0
3.0
2.0
1.0
0
E.K.
W.F.
B.Q.
J.D.
WORK RATE Kgm - m/min
900
800
700
600
CALISTHENICS - WALK - JOG
CONTINUOUS
0 4 8 12 16 20 24
TRAINING TIME (WEEKS)

Figure 14. Changes in exercise heart rate (H.R.) during twenty-four weeks of training at increasing work rates in Groups I, II and III. (Thick line and shaded area group mean ± S.E.).


GROUP I
EXERCISE HEART RATE (beats/min)
190
180
170
160
150
140
130
120
110
100
M.B.
N.S.
L.M.
M.W.
W.S.
WORK RATE (Kgm – m/min)
0
900
800
700
600
CYCLIC
CONTINUOUS
CONTINUOUS
0
4
8
12
16
20
24
TRAINING TIME (WEEKS)


GROUP II
HEART RATE (beats/min)
190
180
170
160
150
140
130
120
110
100
D.B.
H.G.
I.B.
F.C.
H.C.
WORK RATE Kg – m/min
0
900
800
700
600
CONTINUOUS
CYCLIC
CONTINUOUS
0
4
8
12
16
20
24
TRAINING TIME (WEEKS)


GROUP III
HEART RATE (beats/min)
190
180
170
160
150
140
130
120
110
100
W.F.
E.K.
J.D.
B.Q.
WORK RATE Kg – m/min
0
900
800
700
600
CALISTHENICS – WALK – JOG
CONTINUOUS
0
4
8
12
16
20
24
TRAINING TIME (WEEKS)

Circulating Plasma Catecholamines (Rest)

Plasma norepinephrine and epinephrine levels at rest declined from the initial week of training to the final (24th) week of training (norepinephrine .34 ± .04 → .20 ± .03 ng/ml; epinephrine .08 ± .02 → .04 ± .01 ng/ml).

Circulating Plasma Catecholamines (Exercise)

Plasma norepinephrine and epinephrine values during exercise decreased progressively during the training period. During this time as the mean test work rate increased from 639 ± 77 Kg-m/min to 945 ± 63 Kg-m/min, the mean catecholamine levels were observed to rise transiently. As the individuals became accustomed to each increased test work rate the catecholamine levels decreased in subsequent tests at the same work rate. The overall effect was a decrease in the levels of exercise circulating catecholamines (norepinephrine .64 ± .10 → .39 ± .03 ng/ml; epinephrine .18 ± .03 → .09 ± .01 ng/ml) during the twenty-four weeks despite an overall increase in the test work rate.

Oxygen Consumption

Oxygen consumption initially increased slightly with training and remained constant during the last four weeks of continuous training (Figure 13, p. 71).

Heart Rate

The exercise heart rate remained unchanged throughout the training period although the final test work rate was continuously increased (Figure 14, p. 73). The progressive decline in exercise heart rate with

training at a constant work rate of 600 Kg-m/min is shown in Figure 15.

The warm-up heart rate showed a very small decrease over the 24 weeks (initial week 85 ± 5.2 b/min → 24th week 84 ± 4.5 b/min).

Group I showed a constant heart rate, constant oxygen consumption with increasing work rates. Therefore, efficiency of working must be increasing for two reasons (1) because of lower ventilation rate and (2) because of better oxygen extraction. Thus, cardiac output is less and this smaller work of the heart is reflected in decreasing norepinephrine levels.

GROUP II (WORK RATE, CATECHOLAMINES, HEART RATE AND OXYGEN CONSUMPTION)

The mean values for work rate, plasma norepinephrine and epinephrine, heart rate and oxygen consumption for Group II are shown in Table VI. Group II trained initially by continuous effort in bicycling where the load and speed of pedalling remained constant throughout. The power output was maintained at 50% of the currently determined (PWC_{190}) work capacity.

The progress of this group during twenty-four weeks of training is presented graphically in Figures 11-14, pp. 67-74.

Circulating Catecholamines (Rest)

Resting plasma catecholamines declined moderately over the training period (norepinephrine .37 ± .08 → .12 ± .03 ng/ml, epinephrine .10 ± .02 → .06 ± .02 ng/ml).

Figure 15. Changes in exercise heart rate during twenty-four weeks of training at constant work rate of 600 Kg-m/min in Groups I, II and III. (Thick line and shaded area group mean ± S.E.).

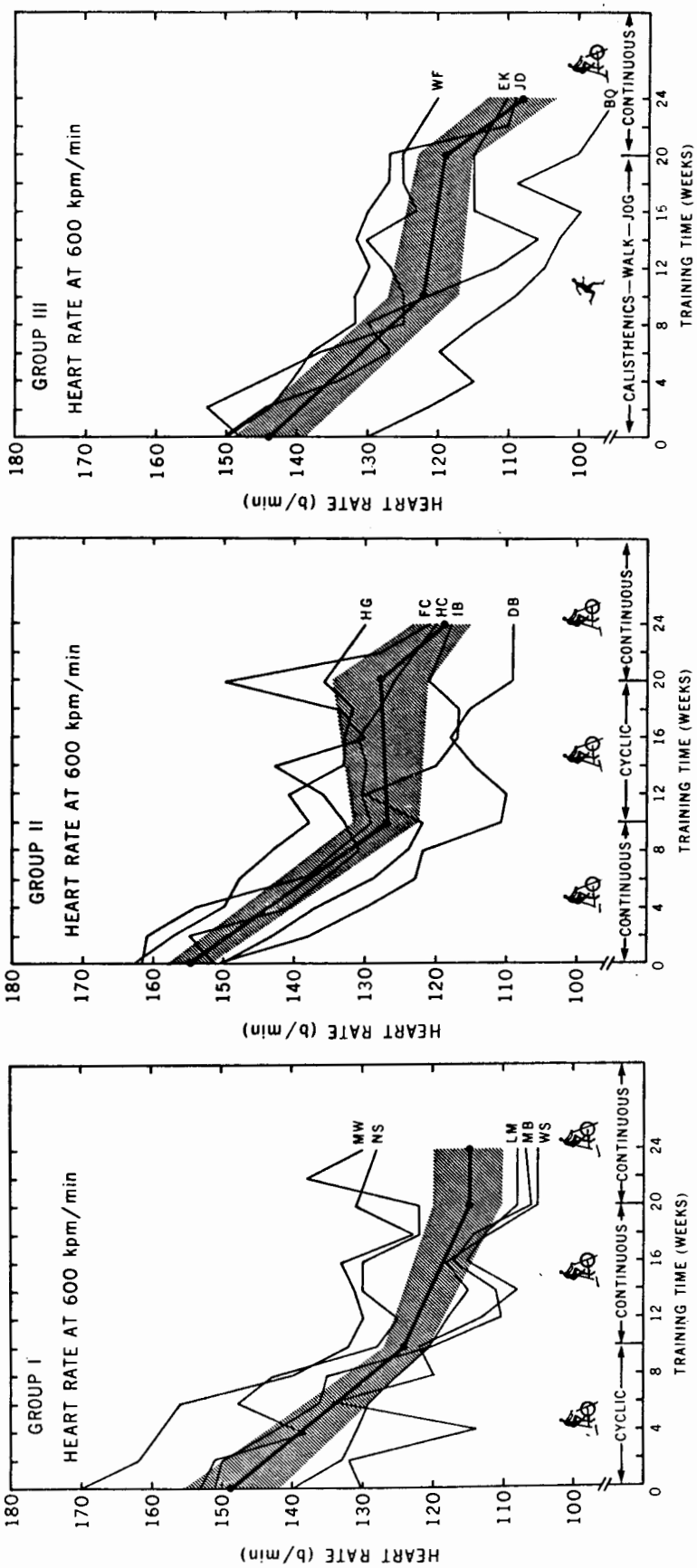

GROUP I
HEART RATE AT 600 kpm/min
MW
NS
LM
MB
WS
GROUP II
HEART RATE AT 600 kpm/min
HG
FC
HC
IB
DB
GROUP III
HEART RATE AT 600 kpm/min
WF
EK
JD
BQ
HEART RATE (b/min)
180
170
160
150
140
130
120
110
100
0
4
8
12
16
20
24
CYCLIC
CONTINUOUS
CALISTHENICS—WALK—JOG
TRAINING TIME (WEEKS)

TABLE VI

WORK RATE (W.R), PLASMA NOREPINEPHRINE (NE), PLASMA EPINEPHRINE (E), HEART RATE (H.R.) AND OXYGEN CONSUMPTION ($\dot{V}O_2$) OF 5 MALE SUBJECTS IN GROUP II DURING A 24 WEEK TRAINING PERIOD (MEAN ± S.E.)

Time (Week)	W.R. (Kg-m/min)	NE (ng/ml)		E (ng/ml)		H.R. beat/min		$\dot{V}O_2$ L/min
		Rest	Exercise	Rest	Exercise	Warm Up	Exercise	
0	535 ± 74	.37 ± .08	.50 ± .09	.10 ± .02	.11 ± .03	91 ± 2.6	142 ± 97	1.78 ± .23
2	570 ± 63	.40 ± .11	.60 ± .09	.09 ± .02	.16 ± .05	90 ± 4.6	147 ± 7.5	1.76 ± .17
4	630 ± 34	.36 ± .13	.55 ± .16	.12 ± .01	.20 ± .04	87 ± 7.8	146 ± 3.1	1.98 ± .15
6	630 ± 34	.38 ± .10	.61 ± .09	.11 ± .02	.19 ± .03	82 ± 5.3	141 ± 3.9	1.78 ± .11
8	690 ± 34	.29 ± .03	.51 ± .06	.10 ± .02	.16 ± .02	79 ± 3.4	148 ± 3.3	2.04 ± .07
10	750 ± 34	.28 ± .04	.46 ± .07	.09 ± .02	.12 ± .05	79 ± 6.6	148 ± 4.6	2.15 ± .11
12	796 ± 34	.24 ± .05	.47 ± .03	.06 ± .01	.18 ± .05	83 ± 4.5	153 ± 3.9	2.18 ± .11
14	796 ± 34	.20 ± .03	.47 ± .08	.07 ± .01	.13 ± .03	76 ± 4.9	146 ± 1.9	2.28 ± .11
16	811 ± 49	.21 ± .05	.48 ± .05	.08 ± .01	.15 ± .04	74 ± 5.3	149 ± 3.8	2.17 ± .12
18	811 ± 49	.22 ± .04	.40 ± .05	.06 ± .01	.13 ± .03	83 ± 4.1	152 ± 3.8	2.25 ± .11
20	811 ± 49	.20 ± .04	.50 ± .06	.09 ± .02	.14 ± .06	77 ± 5.0	148 ± 3.3	2.10 ± .15
22	811 ± 49	.24 ± .04	.42 ± .04	.07 ± .02	.10 ± .02	80 ±11.9	145 ± 2.7	2.13 ± .20
24	827 ± 46	.23 ± .04	.41 ± .09	.06 ± .02	.11 ± .05	78 ± 7.0	141 ± .07	2.08 ± .21

Circulating Catecholamines (Exercise)

Plasma catecholamines (Exercise) decreased with training (norepinephrine .50 ± .09 → .41 ± .09 ng/ml, epinephrine .16 ± .05 (week 2) → .11 ± .05 ng/ml (week 24)).

A more consistent decrease in plasma norepinephrine compared with Group I may be seen both at rest and exercise in Figures 11-14 pp. 67-74). This difference however is not a significant one ($p>.1$).

The epinephrine levels both at rest (.10 ± .02 → .06 ± .02 ng/ml) and during exercise (.20 ± .03 → .11 ± .05) decreased moderately despite increasing work rates in the latter.

There was a significant decrease in circulating catecholamines (epinephrine (rest); norepinephrine (rest and exercise)), during the training period in this group but not significantly greater than in either of the other two exercising groups.

Oxygen Consumption

The oxygen consumption increased over the training period with increasing work rates (1.78 ± .23 → 2.08 ± .21 1/min.).

Heart Rate

Figure 15 (p. 77) shows a decrease in exercise heart rate for a constant work rate of 600 Kg-m/min over 24 weeks of training.

The warm-up heart rates decreased steadily during training (initial week 91 ± 2.6 → 24th week 78 ± 7.0 b/min).

This indicates improved fitness as a result of training and is reflected by the decrease in catecholamine levels.

The mean exercise heart rates at the highest work rates in each test remained between 140 and 150 b/min; they increased transiently as each final test load was initially raised but there was no significant difference noted ($p>.10$) at any stage of training.

GROUP III (WORK RATE, CATECHOLAMINES, HEART RATE AND OXYGEN CONSUMPTION)

Table VII shows the mean values for the work rate, plasma norepinephrine and epinephrine, heart rate and oxygen consumption for Group III. This group trained initially (20 weeks) by calisthenics and jogging. This consisted of thirty-five minutes of a gradual warm-up stretching and flexibility exercises session followed by twenty minutes of a run-walk series. Each subject's progress during the twenty-four week training period is shown in Figures 11-14, pp. 67-74).

Circulating Catecholamines (Rest)

There was a decrease in resting plasma catecholamines over the training period (norepinephrine .28 ± .03 → .17 ± .01 ng/ml; epinephrine .08 ± .01 → .04 ± .01 ng/ml).

A moderate decrease occurred and during the continuous phase of this program a sharper decrease in these levels at a constant work rate of 864 Kg-m/min was observed.

Circulating Catecholamines (Exercise)

During exercise, a definite decline (norepinephrine .44 ± .08 → .32 ± .01; epinephrine .14 ± .04 → .06 ± .01 ng/ml) in the plasma catecholamines occurred, rising transiently only as the final test work

TABLE VII

WORK RATE (W.R.), PLASMA NOREPINEPHRINE (NE), PLASMA EPINEPHRINE (E), HEART RATE (H.R.) AND OXYGEN CONSUMPTION ($\dot{V}O_2$) OF 4 MALE SUBJECTS IN GROUP III DURING A 24 WEEK TRAINING PERIOD (MEAN ± S.E.)

Time (Week)	W.R. (Kg-m/min	NE (ng/ml)		E (ng/ml)		H.R. beat/min		$\dot{V}O_2$ L/min
		Rest	Exercise	Rest	Exercise	Warm Up	Exercise	
0	612 ± 72	.28 ± .03	.44 ± .08	.08 ± .01	.14 ± .04	78 ± 3.9	137 ± 0.7	2.03 ± .18
2	638 ± 83	.30 ± .00	.50 ± .04	.10 ± .00	.13 ± .09	74 ± 4.6	141 ± 1.0	1.82 ± .14
4	675 ± 50	.20 ± .03	.42 ± .03	.08 ± .01	.15 ± .03	82 ± 6.8	146 ± 3.6	1.81 ± .13
6	675 ± 50	.23 ± .00	.43 ± .04	.08 ± .00	.09 ± .00	79 ± 2.1	144 ± 5.7	1.93 ± .22
8	674 ± 41	.22 ± .03	.55 ± .12	.11 ± .02	.18 ± .04	76 ± 4.5	152 ± 4.6	1.90 ± .17
10	731 ± 22	.20 ± .04	.46 ± .11	.07 ± .03	.14 ± .01	74 ± 3.3	141 ± 3.4	2.06 ± .14
12	789 ± 54	.20 ± .02	.42 ± .06	.09 ± .08	.17 ± .04	76 ± 5.8	145 ± 2.2	2.10 ± .25
14	808 ± 42	.22 ± .02	.56 ± .11	.05 ± .03	.10 ± .02	73 ± 5.0	148 ± 3.1	2.13 ± .14
16	808 ± 74	.22 ± .01	.46 ± .04	.08 ± .02	.11 ± .05	70 ± 2.4	143 ± 4.2	2.04 ± .16
18	864 ± 56	.16 ± .03	.37 ± .04	.07 ± .01	.13 ± .05	73 ± 3.5	151 ± 4.8	2.27 ± .17
20	864 ± 56	.23 ± .02	.39 ± .03	.08 ± .01	.12 ± .01	75 ± 7.6	152 ± .07	2.04 ± .15
22	864 ± 56	.18 ± .02	.42 ± .05	.08 ± .01	.13 ± .03	74 ± 5.5	153 ± 4.3	2.12 ± .16
24	864 ± 56	.17 ± .01	.32 ± .01	.04 ± .01	.06 ± .01	80 ± 2.6	148 ± 6.2	2.12 ± .07

rate was increased (612 ± 72 → 864 ± 56 Kg-m/min).

Oxygen Consumption

The oxygen consumption increased in response to the increasing work rate (1.82 ± .14 → 2.12 ± .07 1/min). These oxygen uptakes probably closely represent the individuals' maximum oxygen uptakes.

Heart Rate

At a constant work rate of 600 Kg-m/min, exercise heart rate decreased over the twenty-four week training period in Group III.

The warm up heart rate remained constant during training.

The mean exercise heart rate ranged between 137 and 152 b/min and increased slightly with increasing work rate. Initially the heart rate increased concomitantly with an increased final test work rate but gradually became relatively constant even declining slightly for the same work rate at the end of training (Figure 14, p. 73).

No significant differences between groups were found. However, within the groups there were significant differences between the means of weeks 0-24 for all the groups except epinephrine in the exercise state. ($P<.05$). A significant interaction between Groups I, II and III occurred for the exercise heart rate. Table IV shows these observations. There was a significant decrease in epinephrine circulating concentration (means of all three groups) at rest between the initial week and weeks 10, 18, 20 and 24. This significant decrease also occurred at between 10 and 24 weeks and between 20 and 24 weeks. The level of significance was $p<.10$. Norepinephrine at rest significantly decreased between the initial week

and weeks 18 and 24. This change also occurred between weeks 20 and 24. The level of significance was $p<.01$. In the exercise condition, the level of norepinephrine decreased significantly ($p<.05$) between the initial week and the twenty-fourth week and also between the tenth and twenty-fourth week. The oxygen consumption significantly increased between the initial week and week 24 and between weeks 4 and 18. The level of significance was $p<.01$. The interaction between groups in terms of heart rate can be interpreted from Figure 14, p. 73. In Group I the mean exercise heart rate remains fairly constant during the twenty-four week training period, taking the increasing work rates into account. In Group II there is a slight increase in the exercise heart rate with increasing work rate but a sharp decline is observed during the final continuous phase of this exercise program (weeks 20-24). In Group II a gradual increase in exercise heart rate is observed throughout the first twenty weeks of the calisthenics-walk-jog program. However, when these individuals were changed to continuous bicycle ergometry the heart rate decreased.

Although no significant differences were found between training programs as represented by the three groups, significant changes did occur during the training period of twenty-four weeks. These changes indicated a decrease in norepinephrine and epinephrine levels in training, a decrease in exercise heart rate and an increase in oxygen consumption. These results are explained in the discussion.

CHAPTER V

DISCUSSION

CRITICAL SENSITIVITY OF CATECHOLAMINE ASSAYS

In healthy adult males, the concentrations of epinephrine and norepinephrine in blood plasma are very low; it is, therefore, necessary to use assay techniques of high sensitivity. Great care and patience are required to obtain reliable results. Failure to find an increase in circulating epinephrine by several investigators is probably due to insufficient sensitivity in the assay methods. The EDA method of catecholamine analysis (Gray and Beetham, 1967) indicates this lack of adequate sensitivity, especially for the extremely small circulating epinephrine levels, in that although a consistent three-fold elevation of norepinephrine (NE) within two minutes of severe work (running) was evident, epinephrine (E) elevation was very inconsistent with wide individual variations. Kotchen et al. (1971) used a filter fluorometer and measured human catecholamines by the EDA method. They found no norepinephrine elevation in moderate exercise and a significant elevation of epinephrine only after maximal exercise. These results indicate that the EDA method is not sufficiently sensitive to distinguish small differences in circulating catecholamines. The significant elevation of plasma epinephrine observed in healthy adult males in this study contrasts with the findings of Haggendal et al. (1970) who found a similar

greater increase in arterial norepinephrine concentration during exercise in relation to the relative work levels but little or no increase in circulating plasma epinephrine with increasing work. Vendsalu (1960) and Chin and Evonuk (1971) found increases in plasma norepinephrine after exercise, but no increases in epinephrine levels. It is probable that failure of these investigators described above to find an increase in circulating epinephrine was due to insufficient sensitivity in their methods of analysis.

GENERAL

The current literature is now fairly consistent in describing an increase in plasma catecholamines following exercise in normal adults, the rise being proportional to the amount of exercise performed. However, the effects of long term training on the circulating levels of catecholamines have not been extensively elucidated in normal subjects or post myocardial infarct patients.

CIRCULATING CATECHOLAMINES (REST) IN HEALTHY, NORMAL MALES

The mean resting value for norepinephrine of the five normal healthy males for four work rates was .21 ± .02 ng/ml. The mean resting value for epinephrine was .07 ± .01 ng/ml. These levels reflect the acute response to exercise of normal moderately well trained healthy males currently in regular jogging programs.

CIRCULATING CATECHOLAMINES (EXERCISE) IN HEALTHY, NORMAL MALES

In the group of five normal healthy moderately trained males, elevation of circulating serum catecholamines (venous) increased progressively throughout each work rate and was highest at a work rate of 1800 Kg-m/min. Subsequently catecholamine levels fell progressively but remained elevated up to six minutes post exercise. The lower levels of venous plasma norepinephrine compared to arterial plasma levels reported by Haggendal et al., (1970) may be due to the greater physical fitness of the subjects than those of the normal male adults described here. In addition, the data presented in this study are uncorrected (80% recovery) whereas those of Haggendal et al., (1970) (65% recovery) were corrected.

CIRCULATING CATECHOLAMINES (REST) IN POST MYOCARDIAL INFARCT PATIENTS

The mean initial resting value for norepinephrine (NE) of all fourteen patients (.31 ± .08 ng/ml) was greater than the mean resting value for norepinephrine of the five normal healthy males (.21 ± .02 ng/ml) (ΔNE, $p<.05$). After the twenty-four week training period the mean resting value for NE (24th week) for the total fourteen myocardial patients had reduced to .20 ± .02 ng/ml ($p<.01$).

The mean resting value for epinephrine in the myocardial infarct patients was .08 ± .03 ng/ml which was greater than the mean resting value for epinephrine of .07 ± .01 ng/ml for the group of normal healthy males (ΔE, $p<.05$). After training, the mean resting value for epinephrine in

the myocardial infarct patients had reduced to .05 ± .01 ng/ml (24th week) comparable to the .07 ± .01 ng/ml resting levels of the normal group. This decrease of resting plasma epinephrine was significant ($p<0.01$) (Tables II, V, VI, VII (pps. 55, 66, 79, 82); Figures 8, 9, 11 (pps. 57, 59, 67)).

It seems evident that resting levels of both plasma norepinephrine and epinephrine are reduced with exercise training towards normal values.

CIRCULATING CATECHOLAMINES (EXERCISE) IN POST MYOCARDIAL INFARCT PATIENTS

All fourteen post myocardial infarct patients studied showed a progressive decrease in exercise plasma norepinephrine (NE) (mean initial week .51 ± .12 ng/ml → mean 24th week .37 ± .04 ng/ml) ($p<.05$) and epinephrine (mean initial week .14 ± .03 ng/ml → mean 24th week .09 ± .02 ng/ml) corresponding with training for a period of twenty-four weeks regardless of the type of training followed. The definite nature of this effect is further emphasized by the fact that the overall decrease occurred despite collection of blood samples after the increased final test work rates which were attempted as the patients became trained.

WORK RATE, PLASMA NOREPINEPHRINE (NE) AND PLASMA EPINEPHRINE (E) LEVELS FOR NORMAL SUBJECTS (900 Kgm-m/min) AND FOR POST MYOCARDIAL INFARCT PATIENTS AT THE BEGINNING AND END OF TRAINING

Table VIII shows the mean values for the work rate, plasma norepinephrine and plasma epinephrine for 5 normal healthy subjects at 900 Kg-m/min and for 14 post myocardial infarct patients during the first three weeks

TABLE VIII

WORK RATE (W.R.), PLASMA NOREPINEPHRINE (NE) AND PLASMA EPINEPHRINE (E) OF 5 NORMAL MALE SUBJECTS AND 14 POST MI PATIENTS IN THE UNTRAINED AND TRAINED STATE (MEAN ± S.E.)

State	W.R. (Kg-m/min)	NE (ng/ml)		E (ng/ml)	
		Rest	Exercise	Rest	Exercise
Normal	900 ± 0.0	.24 ± .04	.19 ± .03	.05 ± .01	.07 ± .01
*Untrained (Post MI)	500 ± 35	.31 ± .08	.51 ± .12	.08 ± .03	.14 ± .04
**Trained (Post MI)	909 ± 97	.21 ± .03	.40 ± .05	.07 ± .01	.11 ± .03

*Mean of first three weeks of training.

**Mean of last three weeks of training.

of training (untrained state) and during the last three weeks of training (trained state). The exercise sample was collected at 6 minutes post exercise. A decrease in the levels of both plasma norepinephrine and epinephrine occurred in the post myocardial infarct group with training and at an increased average work load. The final levels approach those for the normal group at a similar work load.

COMPARISONS BETWEEN REST AND EXERCISE AND BETWEEN NORMAL, UNTRAINED POST MI AND TRAINED POST MI STATES FOR NOREPINEPHRINE AND EPINEPHRINE

Table IX compares the resting and exercise values of norepinephrine and epinephrine for 5 normal healthy subjects and 14 post myocardial infarct patients in the untrained and trained states. It also compares the differences between these groups in terms of resting and exercise norepinephrine and epinephrine.

No significant difference was found between resting and exercise in the normal group; at six minutes post exercise the circulating catecholamine levels returned to normal resting values. (Normal values: norepinephrine .25 ± .10, epinephrine .04 ± .04). However, significant differences occurred ($p<.01$) for both norepinephrine and epinephrine in the untrained and trained states of the post myocardial infarct patients. In the untrained group, although resting values were within the normal range, they were still higher than those of the normal group (norepinephrine .31 ± .08 ng/ml: .24 ± .04 ng/ml; epinephrine .08 ± .03 ng/ml: .05 ± .01 ng/ml) and the exercise levels remained considerably elevated (norepinephrine .51 ± .12 ng/ml; epinephrine .14 ± .04 ng/ml). In the untrained

TABLE IX

RESULTS OF ONE WAY CLASSIFICATION ANALYSIS OF VARIANCE, RANDOM DESIGN FOR COMPARISONS BETWEEN REST AND EXERCISE STATES IN NORMAL SUBJECTS, UNTRAINED AND TRAINED POST MYOCARDIAL INFARCT PATIENTS FOR NOREPINEPHRINE (NE) AND EPINEPHRINE (E)

Parameter	Comparison	F Ratio	Degrees of Freedom (df)	Level of Significance
EPINEPHRINE (E)				
Normal	Rest vs Exercise	2.59	1,8	None
Untrained (Post MI)	Rest vs Exercise	9.06	1,26	**
Trained (Post MI)	Rest vs Exercise	9.80	1,26	**
NOREPINEPHRINE (NE)				
Normal	Rest vs Exercise	1.08	1,8	None
Untrained (Post MI)	Rest vs Exercise	8.01	1,26	**
Trained (Post MI)	Rest vs Exercise	66.74	1,26	**
EPINEPHRINE (E)				
Rest	Untrained vs Normal	2.23	2,30	None
	Trained vs Normal	0.05	1,17	None
	Trained vs Untrained	0.01	1,26	None
EPINEPHRINE (E)				
Exercise	Untrained vs Normal	3.50	2,30	*
	Trained vs Normal	0.10	1,17	None
	Trained vs Untrained	2.25	1,26	None
NOREPINEPHRINE (NE)				
Rest	Untrained vs Normal	3.52	2,30	*
	Trained vs Normal	1.32	1,17	None
	Trained vs Untrained	7.29	1,26	*
NOREPINEPHRINE (NE)				
Exercise	Untrained vs Normal	7.27	2,30	**
	Trained vs Normal	79.21	1,17	**
	Trained vs Untrained	2.86	1,26	None

* Significance at .05
df 1,8 F = 5.32
df 1,17 F = 4.45
df 1,26 F = 4.23
df 2,30 F = 3.32

** Significance at .01
df 1,8 F = 11.26
df 1,17 F = 8.40
df 1,26 F = 7.72
df 2,30 F = 5.39

state, the resting value was almost identical with that of the normal group (norepinephrine .21 ± .03 ng/ml: .24 ± .04 ng/ml; epinephrine .07 ± .01 ng/ml: .05 ± .01 ng/ml) and the exercise levels, although not significantly different from those of the untrained post myocardial infarct patients, were less elevated and closer to the normal values (norepinephrine .40 ± .05 ng/ml: .25 ± .10 ng/ml; epinephrine .11 ± .03 ng/ml: .04 ± .04 ng/ml).

No significant differences occurred between normal subjects, untrained and trained post myocardial infarct patients for epinephrine at rest. A significant difference occurred between normal and untrained groups for epinephrine at six minutes post exercise ($p<.05$). However, the differences between trained and normal and trained and untrained groups was not significant for epinephrine at exercise. These results indicate that training reduced the significant difference between the normal subjects and post myocardial infarct patients for epinephrine at six minutes post exercise.

The norepinephrine levels at rest were significantly different between normal and untrained groups ($p<.05$), trained and untrained groups ($p<.05$) but not significant between trained and normal groups, showing that the levels were reduced by training to approximate normal levels. At six minutes post exercise, the norepinephrine levels were significantly different for untrained and normal groups ($p<.01$), and for trained and normal groups but not significantly different for trained and untrained groups. The exercise levels for norepinephrine were reduced by training but still remain elevated as indicated by the insignificant difference between trained and untrained post myocardial infarct patients.

The overall effect of the exercise training was to reduce the high resting and exercise circulating catecholamine levels of the untrained post myocardial infarct patients to normal levels at rest and near normal levels at six minutes post exercise.

METABOLIC EFFECTS OF INCREASED CIRCULATING CATECHOLAMINES

The major mechanism whereby adrenergic stimuli affect glycogenolysis and lipolysis appears to be the stimulation of adenyl cyclase. The catecholamines, epinephrine and norepinephrine both stimulate adenyl cyclase in the liver and in all forms of muscle and this effect in muscle is mediated by β receptors. β adrenergic blocking agents selectively block these effects. In exercise, epinephrine has a more potent effect on metabolism than norepinephrine and the effect of epinephrine is considered to augment 3'5' adenosine monophosphate (cyclic AMP) in liver and skeletal muscle (Lundholm et al., 1966). The role of cyclic AMP as a mediator of adrenergic stimuli is best established for the β metabolic action of the amines, especially the stimulation of skeletal muscle glycogenolysis. Cyclic AMP has been shown to activate a protein kinase that catalyzes the phosphorylation and activation of phosphorylase kinase (Walsh, Perkins and Krebs, 1968). This kinase, in turn, catalyzes the phosphorylation of another protein, phosphorylase b, a reaction that is dependent upon Ca^{++} in micromolar concentration. Phosphorylase b can undergo dimerization and phosphorylation to yield the active α form (Opie, 1968). The phosphorylated α form of this enzyme is the more active in catalyzing the formation of glucose-1-phosphate from glycogen and inorganic phosphate. The protein kinase probably also catalyzes the

phosphorylation of glycogen synthetase, thereby converting the enzyme to the physiologically less active form, synthetase I. This suggests that a single cyclic AMP-stimulated regulatory enzyme controls both the synthetic and degradative pathways of glycogen metabolism. Cyclic AMP mediates the positive effect of epinephrine upon glycolysis lipolysis and gluconeogenesis while suppressing the activity of glycogen synthetase and insulin (Brody and McNeill, 1970; Jost and Rickenberg, 1971; and Robison et al., 1971). Thus, epinephrine might be expected to serve an important role in the provision of substrate for the elevated metabolism accompanying acute physical work. In mammalian sketetal muscle glycogen is undoubtedly an important source of energy for the synthesis of ATP which is subsequently utilized for muscle contraction and epinephrine significantly augments muscle glycogenolysis. It is considered that the effect of glycogenolysis, after augmentation by epinephrine, significantly contributes to the energy metabolism of the mammalian heart. Williams and Mayer (1966) found that a dose of 2.5 ug/Kg min^{-1} infused intravenously in the intact open chested rat produced a net decrease in cardiac glycogen. This dosage caused an activation of phosphorylase and an inhibition of glycogen synthetase. The augmentation of cyclic 3'5'-AMP synthesis by epinephrine mediated these effects.

The hyperglycemia which follows an injection of epinephrine is the result of at least three separate effects:

1) The direct effect on the liver, this results not only in a net increase in the rate of conversion of glycogen to

glucose, but also in a stimulation of the rate at which glucose is synthesized from noncarbohydrate precursors.

2) A direct effect on muscle. This results in an increased rate of conversion of muscle blycogen to lactic acid, which is released into the blood to produce hyperlacticacidemia. Part of the lactic acid is reconverted by the liver to glucose. Thus gluconeogenesis is stimulated by epinephrine not only by its direct effect on the liver but by the increased availability of substrate.

3) A direct effect on the pancreas to suppress the release of insulin which would normally occur in response to the increase in blood glucose levels. This leads to an inhibition of the peripheral utilization of glucose, thus enhancing and prolonging the hyperglycemia which results from the direct effect of epinephrine on the liver.

Thus, epinephrine appears to play an important part in providing substrate for the high metabolic demands of acute exercise. It stimulates adenyl cyclase in the liver and in all forms of muscle. There seems very little reason, therefore, to deny the accumulation of plasma epinephrine during muscular work. Frankenhaeuser et al., (1969) and Docktor and Sharkey (1971) found that the anxiety induced by exercise caused increased levels of urinary epinephrine. Plasma epinephrine levels should be increased similarly under exercise conditions.

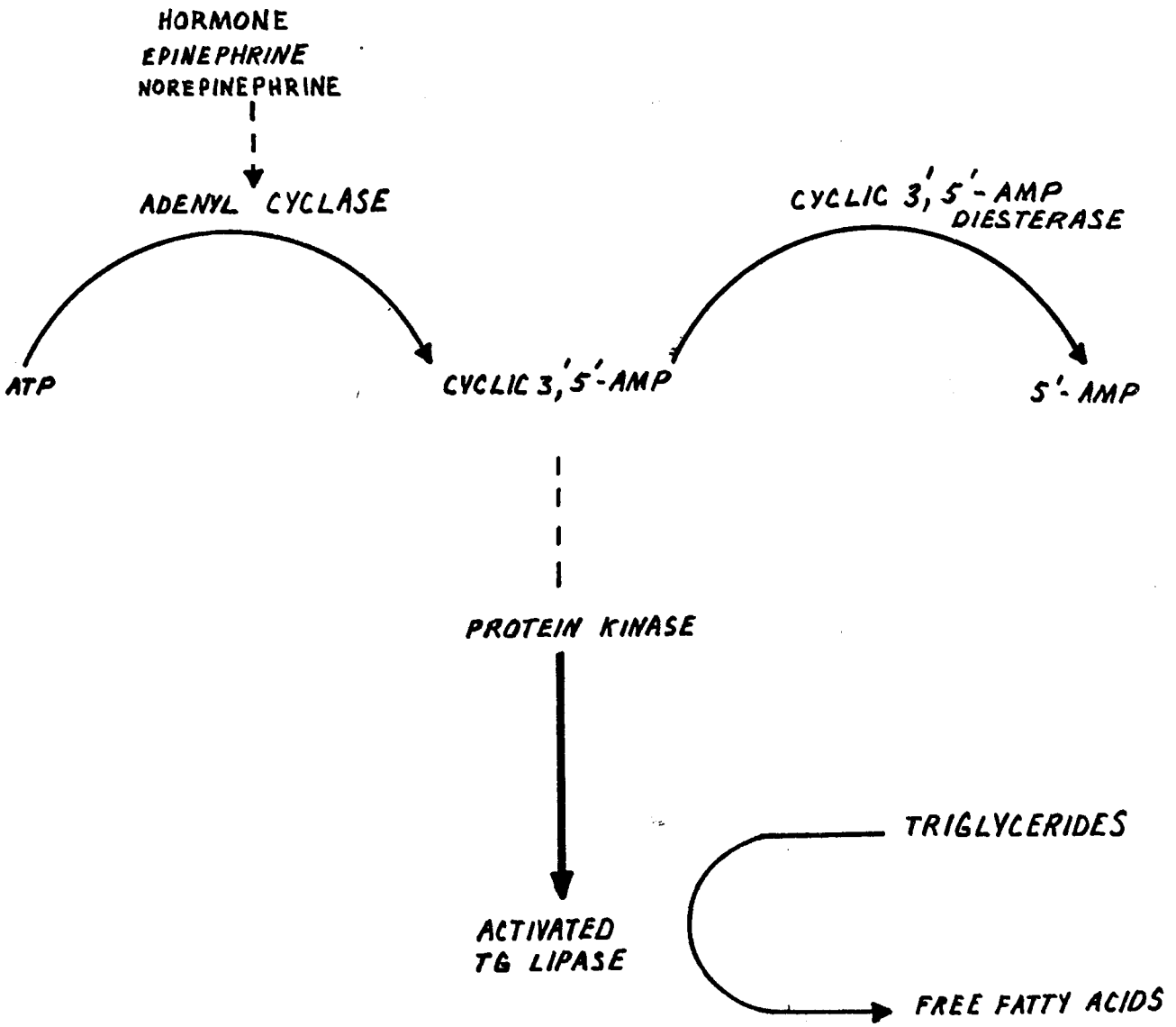
Another biochemical effect of epinephrine is that of lipid degradation. The work of Vaughan and Steinberg (1963) on isolated rat

epidymyal fat pads showed that epinephrine, among a variety of hormones, had a positive effect on lipolysis. Butcher et al., (1965) found that cyclic 3'5'-AMP levels were augmented prior to lipolysis, and Vaughan and Murod (1969) showed that epinephrine activated adenyl cyclase which had been removed from adipose tissue cells. Figure 16 indicates the role played by epinephrine in lipolysis.

Issekutz et al., (1965) demonstrated a greater increase, in trained animals, of free fatty acids during exercise than in untrained animals. Elevations of epinephrine in this study therefore would act to activate triglyceride lipase (Figure 16) (Rizack, 1964; Butcher et al., 1965) which is mediated through cyclic 3'5'-AMP to increase the output of FFA (from adipose tissue) during exercise and this is greatly utilized by working tissues. A comprehensive review of the mobilization and utilization of glycogen and lipids as fuel for muscular activity has been presented by Drummond (1971). This later study has also indicated the need to establish reliable values of the circulating catecholamines during exercise.

For the 5 normal, healthy subjects, a progressive increase in the elevation of the circulating catecholamines occurred throughout each work rate. With increasing work rates and, therefore, with greater demands for metabolic fuel, the plasma circulating catecholamines were more elevated. These elevations indicate that the catecholamines, especially epinephrine, augmented cyclic AMP which mediated the positive effect of the adrenergic stimuli in providing the substrates for the increased metabolic activity.

Figure 16. Effect of epinephrine regulation of lipolysis in adipose tissue.


HORMONE
EPINEPHRINE
NOREPINEPHRINE
ADENYL CYCLASE
ATP
CYCLIC 3,'5'-AMP
CYCLIC 3,'5'-AMP DIESTERASE
5'-AMP
PROTEIN KINASE
ACTIVATED TG LIPASE
TRIGLYCERIDES
FREE FATTY ACIDS

A sustained elevation of the cardiac sympathetic tone with adrenergic preponderance over anti adrenergic parasympathetic and sympathoinhibitory regulatory mechanisms results from habitual sedentary living. This may be concluded from the criteria of a generally accelerated heart rate and shortened isometric tension period (Raab, 1966; Raab et al., 1958) at rest in physically inactive, as compared with active individuals (Kannel, 1967; Raab, 1965; Raab, 1970). Cardiac acceleration during muscular effort is, likewise, greater in sedentary than in trained persons (Brouha, 1959; Frick, 1963; Hollmann, 1963; Kirchoff, 1966; Mellerowicz, 1966; Reindell, 1960) implying a higher and, in part, wasteful oxygen consumption by the heart muscle. Parizková (1969) found that the myocardial concentrations of catecholamines were higher in physically inactive than in trained rats. He suggested that the greater fat content of the non-trained heart muscle may constitute a catecholamine cardiotoxicity-aggravating factor.

Besides sedentary living, it is possible that mental irritations, fear and tensions for example, before competitive events, scholastic examinations, intellectual efforts under pressure, hostile confrontations are associated with cardiac acceleration (Raab, 1966), excessive cardiac oxygen consumption and augmented catecholamine secretion at the terminal nerve ending.

Thus, we find that high levels of catecholamines are associated with physical inactivity and tension. Forssman (1954) has shown that the early post "infarction" period in animals and man is characterized by a regular augmentation of catecholamines in blood and urine. These

findings are further substantiated by the investigations of Hayashi, 1968; Klein, 1966; Miyahara, 1967; Rossini, 1965; Russell, 1958; Sofiyeva, 1965; Sotgiu, 1962; Starcich, 1966 and Valori, 1967.

Since myocardial infarction is associated with high levels of catecholamines, two questions arise:

1) Are high circulating levels of catecholamines, occurring owing to acute stress and lack of physical vigour, precursory conditions inducing the infarction? or
2) Does the heart respond to myocardial infarction by an over-activity of the autonomic system raising the levels of catecholamines in a supportive role?

Sustained infusion of L-epinephrine into the left coronary artery of the intact dog induced myocardial necrosis. Shimamoto (1969) found electron microscope evidence in this condition similar to myocardial anoxia. It seems that any condition producing high levels of catecholamines lead to lesions of the myocardium. In the prenecrotic stage, physical training acts as a cardioprotective mechanism and there is reason to believe that the trained heart is rarely ever infarcted (Raab, 1966).

The sustained antiadrenergic reductions of both heart rate and blood pressure, resulting from habitual physical activity, signify a less oxygen-wasting, more economical utilization of oxidative energy by the heart muscle (Mellerowicz, 1966) and thereby a lessened risk of cardiodestructive hypoxia.

Acutely increased levels of catecholamines have been observed after exercise in normal healthy subjects (Haggendal et al., 1970; Vendsalu, 1960). However, it seems that chronic elevation of catecholamine levels occurs in cardiomegalous conditions (Chidsey et al., 1965). These levels are extremely high compared to those of normal subjects even after exercise stress.

The oxygen consumption for the fourteen post myocardial infarct patients significantly increased (initial mean levels 1.99 ± .28 → final mean levels 2.11 ± .19 L/min) ($p<.01$) with increasing work rates (mean initial 550 ± 35 → mean final 909 ± 97 Kg-m/min). Oxygen consumption for higher work rates increases linearly; however, with training, it may drop slightly at a given submaximal work rate. It is possible that the oxygen uptake measurements noted here were greater than 80% of the maximal oxygen uptake if one considers the magnitude of the accompanying heart rates. Heart rate, on the other hand decreased for a standard submaximal work rate probably reflected reduced demand for blood flow to the working muscle which in itself indicates the enhanced oxygen extractive capability of the tissue.

This effect can be seen from Figure 15 (p. 77) of the heart rate response to standard submaximal work in all three post myocardial training groups and may account in part for the significantly decreased plasma catecholamine levels after exercise.

In this study, heart rate was found to be fairly constant with increasing work rates, rising only slightly at the higher work rates (initial mean 144 ± 4 → final mean 148 ± 4 b/min).

Therefore, the patients were able to consume more oxygen and to do more work at the same heart rate. These results indicate their increase in fitness as a result of the rehabilitative training. No physiologic antiadrenergic measure has proved as effective and as long-acting as rigorous physical training. By contrast, the cardiac functional and metabolic detriments of sedentary living consist chiefly in a loss of adequate parasympathetic and sympathoinhibitory counter regulation against adrenergic, oxygen-wasting and, thus, eventually hypoxiating, adrenergic preponderance in myocardial metabolism.

CHAPTER VI

CONCLUSION

There is an association, moderately close and moderately consistent, between occupational inactivity and the incidence, prevalence and fatality of Coronary Heart Disease (CHD). Although the effect of occupational activity on Coronary Heart Disease is of great theoretical interest, it has a lesser practical relevance. A man cannot be expected to change his job in order to reduce his risk of heart disease; and in any case trends towards mechanization and automation clearly imply that heavy physical work will become increasingly rare. On the other hand, if research on physical activity should support the hypothesis that a short period of moderate exercise each day has a preventative value, then the practical implication of such a finding would be realistic. The heart of the active worker seems better able to survive a myocardial infarction, either because of a better myocardial circulation or else because of some factor of greater myocardial "fitness". Exercise training has been shown to be effective as a therapeutic method. The mechanism of this action includes stimulation of coronary collateral formation and gradual reduction of sympathetic tone.

In this study, elevations of plasma catecholamine levels occurred as the exercise task became more severe for the five normal subjects. In order to cope with the greater metabolic demands induced by the enhanced physical activity, the increase in the catecholamine levels, especially

epinephrine, mediated by cyclic AMP, provided the required metabolic fuel, namely glucose and free fatty acids. The untrained post myocardial infarct patients were found to have high circulating catecholamine levels at rest. It seems probable that because normal activity may represent some degree of stress for these individuals that the high resting catecholamine levels are present in a supportive role to provide substrate for these activities.

At six minutes post exercise, the plasma catecholamine levels for the untrained post myocardial infarct patients were still considerably elevated as compared to normal values for the healthy subjects. Since these normal values represent approximately 25 per cent of the maximum secretory levels during exercise by the five normal males, then the maximum secretory levels for the untrained post myocardial infarct patients must have been very elevated, once again providing the necessary fuels for the tremendous metabolic demand placed on these patients during exercise. After 24 weeks of training, the resting values for the post myocardial infarct patients were normal comparing favourably with the resting values of the normal, healthy group. The six minute post exercise sample, closely approximated the upper values for the normal range and indicated that the maximum secretory catecholamine level was not as high, nor the work task as severe, as in the untrained state. This is even more convincing since the work rates were increased at the end of training. The constancy of heart rate and the slight rise in the oxygen consumption with increasing work rates show the high degree of fitness which these patients achieved.

It is concluded that exercise therapy is a most effective and physiological antihyperadrenergic measure in the rehabilitation of post myocardial infarct patients. By contrast, the detrimental demands of sedentary living consist chiefly in a loss of adequate parasympathetic and sympatho-inhibitory counter-regulation against adrenergic oxygen wasting metabolism.

The results clearly show a decrease in both the resting and exercise plasma catecholamine levels of the post myocardial infarct patients after a 24 week rehabilitative training program. No differences were found between the various patterns of training of the three groups of these patients but a general overall fitness and normalization of circulating catecholamine values at the end of training occurred. They were able to do more work because of a lower ventilation rate, better oxygen extraction and, therefore, less cardiac output or less work of the heart as reflected by the decrease in the plasma catecholamine levels after training.

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APPENDIX

COMPUTER PROGRAM FOR A SPLIT PLOT TWO FACTOR, DESIGN ANALYSIS OF VARIANCE

```
      COMPILER OPTIONS - NAME=  MAIN,OPT=02,LINECNT=54,SIZE=0000K,
                         SOURCE,EBCDIC,NOLIST,NODECK,LOAD,NOMAP,NOEDIT,NOID,
            C**   SPLIT-PLOT TWO-FACTOR DESIGN ANALYSIS OF VARIANCE
            C**   WITH UNWEIGHTED-MEANS SOLUTION FOR UNEQUAL SIZE GROUPS
            C**   LEAD CARD HAS NO. OF ANALYSES IN I4 FORMAT.
            C**   FIRST HEADER CARD FOR EACH SET OF DATA HAS NO. OF LEVELS
            C**   OF FACTOR A, FACTOR B AND TOTAL NO. OF SUBJECTS IN
            C**   I4 FORMAT AND TITLE IN 16A4 FORMAT.
            C**   SECOND HEADER CARD HAS NAMES OF FACTORS A AND B EACH
            C**   IN 10A4 FORMAT.
            C**   DATA FOLLOWS WITH SCORES FOR FIRST SUBJ ON ALL
            C**   LEVELS OF B ON SAME CARD(S)IN FIELDS OF 10
            C**   WITH F10.4 FORMAT.
            C**   DATA IS ORGANIZED AS FOLLOWS  A(1)S(1)B(1)..A(1)S(1)B(J)
            C**   .. A(1)S(K)B(J) ... A(I)S(K)B(J).
            C**   PROGRAM WRITTEN BY J.MONTGOMERY
            C**   REFERENCE-KIRK-EXP.DESIGN-PP245-283
ISN 0002              DIMENSION TITLE(16),FACTA(10),FACTB(10),Y(10,10,20),
                     1SUMS(10,20),SUMSSQ(10,20),AB(10,20),ABSQ(10,20),ABN(10,20),
                     2ABSD(10,20),A(10),B(20),AM(10),BM(20),NS(10),HN(20),FMT(22)
                     3ABVAR(10,20)
ISN 0003              NDEK=0
ISN 0004              READ(5,1)NANAL
ISN 0005        1000  NDEK=NDEK+1
ISN 0006              READ(5,2)NA,NB,NTOT,(TITLE(I),I=1,16)
ISN 0007              READ(5,3)(FACTA(I),I=1,10),(FACTB(I),I=1,10)
ISN 0008              READ(5,4)(NS(I),I=1,NA)
ISN 0009              READ(5,5)FMT
ISN 0010              WRITE(6,98)
ISN 0011              DO 1010 I=1,NA
ISN 0012              NTEMP=NS(I)
ISN 0013              DO 1010 J=1,NTEMP
ISN 0014              READ(5,FMT)(Y(I,J,K),K=1,NB)
ISN 0015              WRITE(6,100)(Y(I,J,K),K=1,NB)
ISN 0016        1010  CONTINUE
ISN 0017              SUM=0.0
ISN 0018              SUMSQ=0.0
ISN 0019              DO 1030 I=1,NA
ISN 0020              NTEMP=NS(I)
ISN 0021              DO 1030 J=1,NTEMP
ISN 0022              SUMS(I,J)=0.
ISN 0023              SUMSSQ(I,J)=0.
ISN 0024              DO 1020 K=1,NB
ISN 0025              SUMS(I,J)=SUMS(I,J)+Y(I,J,K)
ISN 0026              SUMSSQ(I,J)=SUMSSQ(I,J)+Y(I,J,K)**2
ISN 0027        1020  CONTINUE
ISN 0028              SUM=SUM+SUMS(I,J)
ISN 0029              SUMSQ=SUMSQ+SUMSSQ(I,J)
ISN 0030        1030  CONTINUE
ISN 0031              DO 1050 I=1,NA
ISN 0032              NTEMP=NS(I)
ISN 0033              DO 1050 K=1,NB
```

```
ISN 0034              AB(I,K)=0.
ISN 0035              ABSQ(I,K)=0.
ISN 0036              DO 1040 J=1,NTEMP
ISN 0037              AB(I,K)=AB(I,K)+Y(I,J,K)
ISN 0038              ABSQ(I,K)=ABSQ(I,K)+Y(I,J,K)**2
ISN 0039        1040  CONTINUE
ISN 0040              ABM(I,K)=AB(I,K)/NTEMP
ISN 0041              ABVAR(I,K)=ABSQ(I,K)/NTEMP-ABM(I,K)**2
ISN 0042              ABSD(I,K)=SQRT(ABVAR(I,K))
ISN 0043        1050  CONTINUE
ISN 0044              DO 1055 I=1,NA
ISN 0045         1055 CONTINUE
ISN 0046              SSS=0.0
ISN 0047              DO 1060 I=1,NA
ISN 0048              NTEMP=NS(I)
ISN 0049              DO 1060 K=1,NTEMP
ISN 0050              SSS=SSS+SUMS(I,K)**2/NB
ISN 0051        1060  CONTINUE
ISN 0052              SSAA=0.0
ISN 0053              DO 1080 I=1,NA
ISN 0054              A(I)=0.0
ISN 0055              DO 1070 J=1,NB
ISN 0056              A(I)=A(I)+AB(I,J)
ISN 0057        1070  CONTINUE
ISN 0058              SSAA=SSAA+A(I)**2/(NS(I)*NB)
ISN 0059        1080  CONTINUE
ISN 0060              SSB=0.0
ISN 0061              DO 1100 J=1,NB
ISN 0062              B(J)=0.0
ISN 0063              DO 1090 I=1,NA
ISN 0064              B(J)=B(J)+AB(I,J)
ISN 0065        1090  CONTINUE
ISN 0066              SSB=SSB+B(J)**2/NTOT
ISN 0067        1100  CONTINUE
ISN 0068              SSABB=0.0
ISN 0069              DO 1110 I=1,NA
ISN 0070              DO 1110 J=1,NB
ISN 0071              SSABB=SSABB+AB(I,J)**2/NS(I)
ISN 0072        1110  CONTINUE
ISN 0073              HARN=0.0
ISN 0074              DO 1120 I=1,NA
ISN 0075              HARN=HARN+1.0/NS(I)
ISN 0076        1120  CONTINUE
ISN 0077              HARN=NA/HARN
ISN 0078              SSABM=0.0
ISN 0079              DO 1140 I=1,NA
ISN 0080              AM(I)=0.0
ISN 0081              DO 1130 J=1,NB
ISN 0082              SSABM=SSABM+ABM(I,J)**2
ISN 0083              AM(I)=AM(I)+ABM(I,J)
ISN 0084        1130  CONTINUE
ISN 0085        1140  CONTINUE
```

```
ISN 0086              SUMM=0.0
ISN 0087              DO 1150 I=1,NA
ISN 0088              SUMM=SUMM+AM(I)
ISN 0089        1150  CONTINUE
ISN 0090              XM=SUMM**2/(NA*NB)
ISN 0091              X=SUM**2/(NB*NTOT)
ISN 0092              SSAM=0.0
ISN 0093              DO 1160 I=1,NA
ISN 0094              SSAM=SSAM+AM(I)**2/NB
ISN 0095        1160  CONTINUE
ISN 0096              SSBM=0.0
ISN 0097              DO 1180 J=1,NB
ISN 0098              BM(J)=0.0
ISN 0099              DO 1170 I=1,NA
ISN 0100              BM(J)=BM(J)+ABM(I,J)
ISN 0101        1170  CONTINUE
ISN 0102              SSBM=SSBM+BM(J)**2/NA
ISN 0103        1180  CONTINUE
ISN 0104              SSA=HARN*(SSAM-XM)
ISN 0105              SSSA=SSS-SSAA
ISN 0106              SSB=HARN*(SSBM-XM)
ISN 0107              SSAB=HARN*(SSABM-SSAM-SSBM+XM)
ISN 0108              SSBSA=SUMSQ-SSABB-SSS+SSAA
ISN 0109              NDFA=NA-1
ISN 0110              NDFB=NB-1
ISN 0111              NDFAB=NDFA*NDFB
ISN 0112              NDFSA=NTOT-NA
ISN 0113              NDFBSA=NDFSA*NDFB
ISN 0114              AMS=SSA/NDFA
ISN 0115              SAMS=SSSA/NDFSA
ISN 0116              BMS=SSB/NDFB
ISN 0117              ABMS=SSAB/NDFAB
ISN 0118              BSAMS=SSBSA/NDFBSA
ISN 0119              FA=AMS/SAMS
ISN 0120              FB=BMS/BSAMS
ISN 0121              FAB=ABMS/BSAMS
ISN 0122              WRITE(6,6)(TITLE(I),I=1,16)
ISN 0123              WRITE(6,7)(FACTA(I),I=1,10)
ISN 0124              WRITE(6,8)(FACTB(I),I=1,10)
ISN 0125              WRITE(6,9)
ISN 0126              DO 1190 I=1,NA
ISN 0127        1190  WRITE(6,10)(ABM(I,J),J=1,NB)
ISN 0128              WRITE(6,11)
ISN 0129              DO 1200 I=1,NA
ISN 0130        1200  WRITE(6,10)(ABSD(I,J),J=1,NB)
ISN 0131              WRITE(6,12)
ISN 0132              WRITE(6,13)SSA,NDFA,AMS,FA,SSSA,NDFSA,SAMS,
                     1SSB,NDFB,BMS,FB,SSAB,NDFAB,ABMS,FAB,SSBSA,
                     2NDFBSA,BSAMS
ISN 0133              IF(NDEK.LT.NANAL)GO TO 1000
ISN 0135            1 FORMAT(I4)
ISN 0136            2 FORMAT(3I4,16A4)
```

```
ISN 0137          3 FORMAT(2(10A4))
ISN 0138          4 FORMAT(10I4)
ISN 0139          5 FORMAT(20A4)
ISN 0140          6 FORMAT('1',T10,16A4)
ISN 0141          7 FORMAT('0',T10,'FACTOR A IS',10A4)
ISN 0142          8 FORMAT('0',T10,'FACTOR B IS',10A4)
ISN 0143          9 FORMAT('1',T10,'CELL MEANS')
ISN 0144         10 FORMAT('0',4(10X,5(F10.4,10X,/)))
ISN 0145         11 FORMAT('1',T10,'CELL STANDARD DEVIATIONS')
ISN 0146         12 FORMAT('1',T10,'SUMMARY OF SPLIT-PLOT,UNWEIGHTED-
                   1MEANS ANALYSIS',///,T10,'SOURCE',T40,'SUM SQUARES',
                   2T60,'DEGREES FREEDOM',T80,'MEAN SQJARES',T100,'F-RATIOS')
ISN 0147         13 FORMAT('0',T10,'A',T40,F12.6,T60,I4,T80,F12.6,T100,
                   1F10.6,//,T10,'SUBJ.W.A',T40,F12.6,T60,I4,T80,F12.6,
                   2//,T10,'B',T40,F12.6,T60,I4,T80,F12.6,T100,F10.6,
                   3//,T10,'AB',T40,F12.6,T60,I4,T80,F12.6,T100,F10.6,
                   4//,T10,'B X SUBJ W.A',T40,F12.6,T60,I6,T80,F12.6)
ISN 0148         98 FORMAT('1',10X,'CALCULATIONS')
ISN 0149         99 FORMAT(10I12)
ISN 0150        100 FORMAT(10F12.4)
ISN 0151            STOP
ISN 0152            END

OPTIONS IN EFFECT*      NAME=  MAIN,OPT=02,LINECNT=54,SIZE=0000K,

OPTIONS IN EFFECT*      SOURCE,EBCDIC,NOLIST,NODECK,LOAD,NOMAP,NOEDIT,NOID,NOXRE

STATISTICS*     SOURCE STATEMENTS =     151 ,PROGRAM SIZE =    18304

STATISTICS*  NO  DIAGNOSTICS GENERATED

***** END OF COMPILATION ******                                    71K BYTES
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