EXERCISE AND THE HEART.

EFFECTS OF EXERCISE TRAINING ON CORONARY ARTERY DISEASE, AND ON MYOCARDIAL FUNCTION, METABOLISM, AND VULNERABILITY TO VENTRICULAR FIBRILLATION.

A Thesis submitted to the University of Cape Town

for

The Degree of Doctor of Medicine

by

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DEDICATION.

For my parents Bindy and Wendy, and my wife Marilyn Anne, with love and gratitude expressed, as always, imperfectly.

Cape Town

February, 1981

FOREWORD.

"Is there any danger with an athletic heart?

The main danger is going to see the doctor."

GEORGE SHEEHAN M.D.

DR. SHEEHAN ON RUNNING (1975).

ACKNOWLEDGEMENTS.

It is with very real pleasure and deep gratitude that I acknowledge the substantial contributions made by a number of people to the completion of this thesis.

To my parents and to my wife, to all of whom I dedicate this thesis, I am the most indebted. Collectively, they provided the supportive environment that was absolutely fundamental to the completion of this work. My parents must be especially thanked for providing the financial security that allowed me to follow my academic interests unreservedly. In addition I was fortunate to have parents who have never expressed anything but their total support and encouragement for whatever I have personally chosen to do. My wife Marilyn has borne the agonies of this thesis with a degree of equanimity and patience that far exceeds anything expected by the marriage vows. Without the support, understanding and encouragement of this most complete person, there would simply have been no thesis, and no work of any substance.

It was an extreme privilege for me to have worked with, and in the laboratories of, Professor L.H. Opie. He provided an exceptional example of quite superhuman, tireless energy and an insatiable desire for perfection. I am grateful for the unique opportunity I have enjoyed in being exposed to his brilliant, inquisitive mind and to have observed, at close hand, his outstanding lecturing, teaching and writing skills. Above and beyond all these special attributes, Professor Opie helped procure research funds and provided the laboratory facilities in which I learned to use the isolated perfused working rat heart model - a model, to the historical development of which he has contributed. Finally, I am thankful for the unbridled freedom with which Professor Opie allowed

me to pursue all my research and academic interests.

In the five year period during which this research was performed, I received financial assistance from the South African Medical Research Council and the Guy Elliott Memorial Research Fellowship. I thank these granting bodies for their trust, which has allowed me to complete this work and to embark on a career which I have found so interesting, exciting and personally rewarding.

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DECLARATION.

This thesis is the original work of the author, both in its concept and execution. The results of the work and ideas of others mentioned in the text are fully referenced. Where data, collected by others in our collaborative studies, has been reproduced, this has been with their full permission and I have fully acknowledged the source of such data.

Portions of the work described in this thesis have already been published:-

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Journal of Molecular and Cellular Cardiology, 13, 753-765, 1981.

T.D. Noakes.

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ABSTRACT.

There is epidemiological and experimental evidence suggesting that exercise training may reduce the mortality rate from coronary heart disease, in particular the sudden death rate, and that it may improve the peak functional capacity of the heart. This thesis includes experimental work that is relevant to both these questions.

EXERCISE AND CORONARY ATHEROSCLEROSIS. STUDIES OF CORONARY HEART DISEASE IN MARATHON RUNNERS.

To determine whether exercise training can prevent coronary atherosclerosis, I chose to establish whether coronary heart disease could be found in marathon runners.

Interest in this particular group of sportsmen stems directly from a letter published in the Lancet in 1972, which proclaimed that "a search of the literature by the American Medical Joggers Association failed to document a single death due to coronary atherosclerosis amongst marathon runners". The implication of this statement was that the high levels of habitual physical activity maintained by marathon runners must be sufficient to prevent the development of coronary atherosclerosis, or, alternatively, to increase myocardial resistance to those factors promoting ventricular fibrillation in persons with coronary heart disease. A third possibility was that coronary heart disease does indeed exist in marathon runners, but that no previous work has been sufficiently rigorous to find such persons.

To distinguish between these possibilities, a survey of heart disease amongst South African marathon runners was instigated. During a five-year period, adequate clinical material was collected on 5 cases

of acute myocardial infarction and 3 cases of sudden death in South African marathoners. Three runners with acute myocardial infarction had coronary angiographic evidence of coronary atherosclerosis whereas all 3 runners who died suddenly had this disease proven by autopsy. Of another 3 runners killed in a motor car accident, 2 had autopsy-proved coronary atherosclerosis. These findings have therefore disproved the hypothesis that marathon running alone can provide complete immunity to coronary atherosclerosis.

Important additional points that arose from this study were that:

1) All runners developed warning symptoms prior to their subsequent heart attacks or sudden deaths.

2) In these runners, high levels of physical fitness did not guarantee cardiovascular health.

3) The possibility that overtraining or overcompetitiveness had been a factor precipitating the onset of myocardial infarction, sudden death or angina pectoris in some of these runners must be considered.

It must be emphasized that these individual case studies provide no information on whether coronary disease and cardiovascular mortality are more or less frequent in the overall group of marathon runners. Nor do they detract from the more general thesis that marathon running or other forms of vigorous exercise might provide partial protection against coronary atherosclerosis or sudden cardiac death. The ultimate resolution of these questions will not come from individual case studies, but must await the results of major epidemiological studies.

EXERCISE TRAINING AND MYOCARDIAL RESISTANCE TO VENTRICULAR FIBRILLATION. STUDIES IN THE ISOLATED PERFUSED RAT HEART MODEL.

To study further the alternate possibility that exercise training might increase resistance to sudden death by increasing myocardial resistance to ventricular fibrillation, the ventricular fibrillation thresholds of hearts from trained and control rats were studied in the isolated perfused heart model. Ventricular fibrillation thresholds were determined in hearts from trained and control rats during control perfusions, during acute regional ischaemia, during hypoxia and during hypoxia combined with isoproterenol infusion.

Under all perfusion conditions, hearts from trained animals had significantly higher ventricular fibrillation thresholds. During acute regional ischaemia, cyclic AMP levels in the ischaemic left ventricular zones were significantly lower in trained hearts.

These data are therefore in accord with the epidemiological evidence which suggests that physically-trained humans have increased resistance to sudden coronary death.

MYOCARDIAL ADAPTATIONS TO EXERCISE TRAINING. PART I. FACTORS CONTROLLING MAXIMUM HEART FUNCTION IN THE ISOLATED PERFUSED RAT HEART MODEL.

To study the nature of the myocardial adaptations to exercise training, hearts from trained and control rats were studied in the isolated perfused working rat heart model. But, because there is evidence that the myocardial adaptations to exercise training are only apparent when the heart is maximally stressed, it was first necessary to determine the perfusion conditions that elicit peak function in the

isolated rat heart model, as these conditions have not previously been determined.

It was found that peak heart function was achieved when hearts, paced at heart rates between 330 and 390 beats/min, were perfused at an atrial filling pressure of 25 cmH_2O with an aortic column height of 120 cm. Under these conditions, stroke volumes and calculated heart work were substrate-dependent and were lowest in hearts perfused with 1 mM palmitate or with 10 mM pyruvate and 11,1 mM D(+)-glucose plus insulin at 2 u/litre or with 11,1 mM glucose or with 10 mM L(+) lactate, and were highest in hearts perfused with either 11,1 mM glucose plus insulin (2 u/litre) or 11,1 mM glucose plus 10 mM lactate plus insulin (2 u/litre).

Studies of left ventricular pressure changes showed that the addition of insulin to glucose or of glucose/insulin to lactate or palmitate in the perfusion fluid resulted in increased stroke volumes, heart work, peak left ventricular systolic pressures and maximum rates of both left ventricular pressure development (LV max +ve dP/dt) and left ventricular relaxation (LV max -ve dP/dt).

Substrate-induced differences in stroke volumes measured under maximum perfusion conditions could not be explained on the basis of differences in the LV max +ve dP/dt values because the highest LV max +ve dP/dt values were recorded in hearts perfused with 10 mM pyruvate and 11,1 mM D(+) glucose plus insulin (2 u/litre), in which hearts, stroke volumes were amongst the lowest recorded. Rather, differences in stroke volumes amongst hearts perfused with the different substrate combinations could best be explained on the basis of substrate induced differences in the rates of left ventricular relaxation (LV max -ve dP/dt), suggesting that diastolic phenomena may determine stroke volumes in this model.

A significant linear correlation was found between the mean

maximum rates of left ventricular relaxation and the mean rates of glycolytic ATP production of hearts perfused with the various substrate combinations. Further evidence for this relationship was the finding that the specific glycolytic inhibitor, deoxyglucose, had a specific effect on the rate and duration of myocardial relaxation. De-oxyglucose did not alter myocardial oxygen consumption rates, suggesting that its metabolic effect was specific to glycolysis and did not influence rates of oxidative ATP production.

These studies suggest that the rate of myocardial relaxation is an important factor determining stroke volume in the isolated perfused rat heart model. Furthermore, they establish a relationship between the rates of myocardial relaxation and the rates of glycolytic ATP production, suggesting that glycolytically-produced ATP may play a special role in the energetics of myocardial relaxation. This would best be explained on the basis of cellular compartmentalization which necessitates the utilization of glycolytic ATP for the re-uptake of myoplasmic calcium by the sarcoplasmic reticulum during diastole.

Additional findings not directly relevant to this thesis were that:

1) The presence of a catheter in the left ventricle of the isolated working heart impairs its performance.

2) There are substrate-induced differences in myocardial efficiency.

3) The presence of albumin in the perfusion fluid alters the fluid viscosity sufficiently to reduce stroke volume and therefore calculated heart work and myocardial efficiency.

MYOCARDIAL ADAPTATIONS TO EXERCISE TRAINING. PART II. EFFECTS OF RUNNING TRAINING ON MYOCARDIAL METABOLISM AND FUNCTION.

Physical training (running on an inclined motor-driven treadmill for up to 2 hours a day, 5 days a week for 8 weeks) had 4 principal effects

on myocardial metabolism and function studied under maximum conditions in the isolated perfused working rat heart model.

First, under steady state perfusion conditions, there were no differences in heart function between trained and control rats. These differences only became apparent either when hearts were perfused under conditions of changing atrial filling pressures and heart rates and with isovolumic beats interspersed or, when the β-agonist isoproterenol was infused. Under steady perfusion conditions and with $CaCl_2$ concentration of 2,2 mM, isoproterenol infusion caused peak left ventricular systolic pressures and both LV max +ve and max -ve dP/dt values to be significantly greater in hearts from trained animals perfused with 11,1 mM glucose plus insulin (2 u/litre). When isoproterenol was infused at higher, unphysiological calcium concentrations (3,6 mM), there were no differences in mechanical function between hearts from trained and control animals. This finding provides strong evidence that the training-induced adaptations in heart function are mediated by calcium.

Second, hearts from trained rats had significantly greater rates of myocardial glycolytic ATP production during both control perfusion and after isoproterenol infusion. Higher rates of myocardial glycolysis could therefore not explain the increased stroke volumes measured in trained hearts after isoproterenol infusion, because glycolytic rates were also higher during control perfusions under which conditions stroke volumes were not different between the groups.

Third, trained hearts had significantly higher myosin Ca^{++}-ATPase activities during both control perfusions and after isoproterenol infusion. Levels of myosin P light chain phosphorylation were also increased in trained hearts. Both these differences were magnified at the higher perfusate $CaCl_2$ concentration and were not related to differences in myocardial cyclic AMP levels. From these results the following is concluded:

1) The increased mechanical function and the increased myosin Ca^{++}-ATPase and myosin P light chain phosphorylation levels measured in trained hearts all result from a training-induced increase in trans-sarcolemmal calcium transport capacity.

2) Peak heart function during β-stimulation is achieved at a certain level of intracellular calcium (and therefore a certain level of myosin P light chain phosphorylation and Ca^{++}-ATPase activity) and that further increases in these parameters cause no further increase in left ventricular function.

ABBREVIATIONS.

ADP	Adenosine 5' diphosphate
ADP/O ratio	Adenosine 5' diphosphate to oxygen ratio
AMP	Adenosine 5' monophosphate
ATP	Adenosine 5' triphosphate
ATPase	Adenosine triphosphatase
β-	Beta-
Ca^{++}	Calcium ion
$CaCl_2$	Calcium chloride
CF	Coronary flow rate
cm	centimetres
CO_2	Carbon dioxide
C(P)K	Creatine (Phospho)kinase
CPM	Counts per minute
Cyclic AMP	3',5' cyclic adenosine monophosphate
Cyclic GMP	3',5' cyclic guanosine monophosphate
D(+)	Dextrorotatory: D configuration
DNA	Deoxyribonucleic acid
$(dP/dt)p^{-1}$	contractility index
EDP	End diastolic pressure
EDTA	Ethylene diaminetetra-acetate
ESR	Erythrocyte sedimentation rate

g or gm	grams
G6P	Glucose 6-phosphate
G6PDH	Glucose 6-phosphate dehydrogenase
H^+	Hydrogen ion
Hr	hour
H_2O	Hydrogen$_2$ oxide (water)
HDL-cholesterol	High density lipoprotein fraction of cholesterol
Hz	Hertz
IU	International Units
K^+	Potassium ion
Kcal	Kilocalories
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
Km	kilometer(s)
KOH	Potassium hydroxide
L(+)	Levarorotatory: D configuration
l	litre
LDH	Lactate dehydrogenase
LV	Left ventricular
LVET	Left ventricular ejection time
L_{max}	Muscle length at which maximum isometric tension is developed

LV max +ve dP/dt	Maximum rate of left ventricular pressure development
LV max -ve dP/dt	Maximum rate of relaxation (left ventricular pressure fall)
M	Molar
mA	milliamperes
Max dT/dt	Maximum rate of tension development
MDH	Malate dehydrogenase
mg	milligrams
Mg^{++}	Magnesium ion
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
min	minute
ml	millilitre
mM	millimolar
mmol	millimole
mmHg	millimetres of mercury
MVO_2	Myocardial oxygen consumption rate
n	nano
Na^+	Sodium ion
NaCl	Sodium chloride
NAD^+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced form)
$NADH_2$-Na_2	Sodium salt of reduced nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate

NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NaOH	Sodium hydroxide
$NaHCO_3$	Sodium bicarbonate
N_2	Nitrogen
O_2	Oxygen
PCA	Perchloric acid
PCO_2	Partial pressure of carbon dioxide
PCr	Phosphocreatine
PDT	Peak developed tension
PEP	Pre-ejection phase
Pi	Phosphate
pm	post meridian
P/O	Phosphorus/oxygen ratio
P_o	Maximum developed force
PO_2	Partial pressure of oxygen
QO_2	Rate of tissue oxygen consumption
$Q\text{-}S_2$	Duration of electromechanical systole
RCI	Respiratory control index
RIA	Radioimmunoassay
RNA	Ribonucleic acid
$\frac{1}{2}RT$	Time for tension to fall to one - half peak developed tension
rpm	revolutions per minute

SGOT	Serum glutamate oxaloacetate transferase
SV	Stroke volume
TDT	Time to peak developed tension
Tris buffer	Tris-(hydroxymethyl)-aminomethan
u	units
Vmax	Idealized maximum shortening velocity with zero load
VO_2max	Maximum rate of (whole body) oxygen consumption
µ	micro

I N D E X

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CHAPTER 1.

INTRODUCTION TO, AND SCOPE OF THE THESIS.

There are 3 reasons why I chose to study the effects of exercise on the heart for this thesis: First, my lifelong obsession with sport; second, the moulding that resulted during my years as a medical student of this obsession into a dispassionate interest in the medical and scientific aspects of sport; and third, my exposure to Professor L.H. Opie and the opportunity to study the effects of exercise on the heart in his laboratory.

I justified the viability and scientific relevance of this topic on the basis of what I perceived to be a widespread medical ignorance, influenced by strongly prejudicial beliefs, of the effects of exercise on the heart. In the writings of Dr. George Sheehan, a cardiologist, now a personal friend, I was to discover the critical support for this embryonic opinion. If, as Dr. Sheehan has written (see Foreword), "the main danger (of the athletic heart) is going to see the doctor", then clearly any studies that could alter this would be both helpful and timely.

Whilst a medical student, I began to read the sports medical literature, paying particular attention to those studies relating to exercise and the heart. It soon became apparent that 2 principal areas of research had been identified. The first and most obvious question was whether exercise plays a role in preventing mortality from coronary heart disease, a disease which extracts such an enormous toll in this country. If exercise training does indeed have such a protective effect, it raises the further question of whether this effect is due either to a direct effect of exercise training on the myocardium or to an independent effect (for example, on either the blood, or the central or autonomic nervous systems).

The second question identified in the literature, which had clearly not been finally resolved, was the physiological and biochemical nature

of the myocardial adaptations to training, if such adaptations do in fact occur.

Chapter 2 of this thesis provides a review of all the published literature which I consider to be relevant to these 2 questions. The reader may find it long and detailed, but I was unable to find a single source, or collection of sources, that thoroughly reviewed all this information in the manner I have set out. I would hope that Chapter 2 will provide future researchers in this field with a fairly concise review of the literature, sparing them the agonies which I endured, and which I fear still show, in the compilation of that chapter.

In the original work performed by me, and which begins in Chapter 3, I was fortunate in having the opportunity to perform studies that relate to both the major research questions identified above.

First, concurrent with my first years as both a medical student and a marathon runner, I became aware of the hypothesis proposed by the American pathologist Dr. Tom Bassler which stated, in essence, that if one did sufficient exercise, specifically if one trained enough to complete a 42 km marathon running race, then one became immune to coronary atherosclerosis. It is not difficult to understand why this was a very exciting hypothesis for me, as both a marathon runner and a fledgling "scientist". Not only did it have direct bearing on my sport, but it had the added attraction that it would be fairly easy to study.

For this reason I began an on-going study to determine whether coronary atherosclerosis could be found in active marathon runners who fulfilled the criteria dictated by the "Bassler hypothesis". Chapter 3 describes the current status of this work, which shows that not even the very high levels of physical activity maintained by most marathon runners will guarantee absolute immunity against either myocardial infarction,

coronary atherosclerosis or sudden coronary or other cardiac death.

But even to this most ardent runner and naive scientist, it had always seemed unlikely that Dr. Bassler's extreme postulate could be possible, and that the truth would almost certainly be found in a more moderate hypothesis. But to evaluate such an hypothesis further would have required massive epidemiological studies, for which I have no facilities at present.

To continue this work, I chose therefore to do relevant studies in an animal preparation, the isolated perfused rat heart model. The studies were initially designed to establish whether exercise training improves myocardial electrical stability, in particular during those experimental interventions that are believed to mimic conditions present during heart attack. These studies are described in Chapter 4.

Chapter 5 introduces my studies of the second relevant question, namely, the myocardial metabolic and functional adaptations to exercise training. That chapter describes the methodology of the initial studies designed to improve the isolated perfused working rat heart model, so that it could be used more effectively to study the myocardial adaptations to exercise training. These initial studies were essential because the perfusion conditions necessary to elicit maximum heart function are not known, and the indications in the literature are that the myocardial adaptations to exercise training become apparent only when the heart is maximally stressed. In the course of these studies a number of additional conclusions, not directly relevant to the main topic of this thesis, were identified. These are nevertheless included in this thesis, either because they are relevant to

the question of heart metabolism and performance during maximum work (findings which are presumably applicable to what happens during maximum exercise) or because they will be of interest to other workers who use the isolated perfused working rat heart model.

Chapter 6 reports my studies of the effects of exercise training on myocardial performance and metabolism studies under the maximum conditions identified in Chapter 5. It includes biochemical data on contractile protein ATP hydrolysing activities and phosphorylation levels collected by Miss Thérèse Resink and Professor W. Gevers in our collaborative studies. Their data are included with their permission in this thesis, because they provide additional critical evidence to explain the biochemical basis for the cardiac adaptations to exercise training.

Chapter 7 concludes this thesis by providing a final summary and criticism of this work. It provides an overview of how these studies relate to the published literature, and suggests avenues for further research.

CHAPTER 2.

REVIEW OF STUDIES REPORTING THE EFFECTS OF EXERCISE TRAINING ON CORONARY ARTERY DISEASE, AND ON MYOCARDIAL FUNCTION, METABOLISM, AND RESISTANCE TO VENTRICULAR FIBRILLATION AFTER TRAINING.

"..... the conscientious inquirer into the effects of violent exercise is bewildered by discrepancies and contradictions".

Sir Adolphe Abrahams.
Arris and Gale lecture on the physiology of violent exercise in relation to the possibility of strain.
Lancet 1, 429-435, 1928.

2.1 HISTORICAL BACKGROUND.

The classical writings include numerous references postulating a relationship, beneficial or otherwise, between physical activity and health.

The Homerian concept of a healthy body in a healthy mind - mens sana in corpore sano - was captured in the writings of Plato (428-348 BC) who was himself a top-level amateur wrestler. In Timeaus and Cortias[1], he wrote that "avoid exercising either mind or body without the other, and thus preserve an equal and healthy balance between them. So anyone engaged on mathematics or any other strenuous intellectual pursuit should also exercise his body and take part in physical training". Cicero[2] (106-43 BC) preached that "exercise and temperance can preserve something of our early strength even in old age", while Socrates is reputed to have said: "It is disgraceful for a person to grow old in self-neglect before he knows what he would become by rendering himself well-formed and vigorous in body"[3].

But the Greeks did not view exercise purely as a means of maintaining physical health. In the words of Kitto[4]: "The Greek made physical training an important part of education, not because he said to himself, 'Look here, we mustn't forget the body', but because it could never occur to him to train anything but the whole man". For the Greeks then, exercise was essential for training the whole man, and their sporting contests were designed to test the arête - the excellence - of the whole man.

With the rise of the Roman Empire, these Graecian values were lost as the affluent Romans turned to those spectator sports - chariot races, gladiatorial combats, and fights with wild animals - which they had learned from the Etruscans. Ultimately these activities held in arenas such as the Circus Maximus (seating capacity - 385 000) degenerated to the point

where they became simply a means of buying the popular votes[5].

Partly as a result of this debasement of sport by the Romans, there was a move away from physical activity, lasting right up to the Renaissance. The responsibility for at least part of this probably lies with the Church, whose bias is suggested in the writing of Paul: "Exercise thyself rather into godliness. For bodily exercise profiteth little: but godliness is profitable unto all things, having promise of the life that now is, and of that which is to come"[6].

But the Renaissance, broadly described as that period of Western History between the 14th and 17th century, brought with it a renewed appreciation of the important role exercise plays in spiritual and physical health. Martin Luther (1483-1546) himself spoke of the recreative and moral aspects of physical exercise. In Britain, Henry VIII strongly approved of exercise and in his youth played tennis daily. In 1618, James I promulgated the "Declaration of Lawful Sport" which allowed the labouring classes to play sport on Sundays. All the major Utopian writers of this era stressed the importance of regular physical activity[7]. In his Utopia, More envisaged a society in which "nowhere else are men's bodies more vigorous and subject to fewer diseases". In Campanella's Civitas Solis (the City of the Sun), sedentary games were prohibited and everyone participated in therapeutic physical activity as it was believed that much disease was due to lack of exercise. In Hartlib's Macaria, each member of the community was charged with responsibility for both his soul and his body, whereas in Nova Solyma (Jerusalem Regained) Samuel Gott re-affirmed the Greek belief that the good in life depended on a healthy body. Exercise was mandatory and from their earliest years, the children of Jerusalem learned to endure physical hardship. They were taught to "play", and to value the pleasure of participation rather than "the money value of their athletic prizes".

British poets of this era also emphasized the importance of regular exercise. In his essay "Of education" John Milton (1608-1674) recommended that boys exercise for 3½ hours a day, whilst in the poem "To John Driden of Chesterton", Dryden (1631-1700) wrote:

"Better to hunt in fields for health unbought,
Than fee the doctor for a nauseous draught.
The wise, for cure, on exercise depend;
God never made his work, for man to mend".

In passing, it is of interest to record that many of the world's great poets and thinkers including Nietzsche, Kant, Kierkegaard, Thoreau, Emerson, Jefferson, Wordsworth and Coleridge, were prolific walkers.

Ninety years after Dryden's poetic observations, Easton[8] published the first scientific documentation suggesting a link between physical activity and increased longevity. From a study of 1712 instances of longevity greater than 100 years, he concluded that "it is not the rich and the great, nor those who depend on medicine who become old: but such as use much exercise. For an idler never attains a remarkable great age".

More recent studies confirm an association between longevity and regular, lifelong physical activity. In a review of 52 British centurians published in 1889, Humphrey[9] observed that these men were persistently physically active, particularly out-of-doors. As his first of 12 precepts for longevity, Lorand[10] wrote in 1911: "To be much as possible in the open air, and especially in the sunshine; and to take plenty of exercise, taking special care to breath deeply and regularly". More recently, both Belloc[11] and McGlone and Kick[12] have observed an association between regular physical activity and increased longevity.

In contrast to these generally favourable attitudes, the medical profession has not always been quite so well-disposed to exercise. Although

both Hippocrates (460-375 BC) and his illustrious successor Galen (130 -201 AD) prescribed remedial exercises for the treatment of a variety of medical conditions, both were vigorously opposed to strenuous activity. In his treatise on nutriment, Hippocrates warned that "the condition of the athlete is not natural. A healthy state is superior to all". Galen was even less complimentary. In his Paraphrase of Menodotus, he proposed that "athletes live a life quite contrary to the precepts of hygiene, and I regard their mode of living as a regime far more favourable to illness than to health.... When they give up their profession, they fall into a condition more parlous still; as a fact, some die shortly afterwards; others live for some little time, but do not arrive at old age".[13]

Evidence that this attitude survived the Renaissance is provided by a letter published little more than a century ago, on October 11th, 1869 in the Times of London. The letter's author, Frederick C. Skey FRCS, a former President of the Royal College of Surgeons, charged that "the University Boat Race (the rowing race on the Thames contested annually by Oxford and Cambridge) as at present established is a national folly". He claimed that rowing was bringing young University oarsmen to an early grave and was "destroying the ephemeral bloom of these apparently stalwart champions of the oar".

The letter aroused heated controversy in the correspondence columns of the Times leading to an Editorial which with creditable impartiality, pointed out that no statistical inquiry into the subject had ever been made, and until that was done no firm conclusions could be arrived at.[13]

An important consequence of this Editorial was that it stimulated Dr. John Morgan, a Birmingham physician and former University oarsman, to undertake the first scientific study to determine whether strenuous physical activity in youth did in fact influence subsequent longevity.[14] Morgan traced 200 of that group of oarsmen who had rowed in the University Boat Race

between 1829 and 1869, and compared their longevity to that of the "average" insured Englishman, a figure obtained from Dr. Farr's "English Life Tables". The completed study refuted the contention that strenuous exercise in youth leads to an early death, because the calculated longevity of the oarsmen was 2 years greater than Dr. Farr's "average" Englishmen. Despite methodological criticisms of his work[13,15], in particular criticism of the small numbers studied and the inadequate control group, Morgan's essential findings that vigorous athleticism in youth does not reduce longevity, has been confirmed by many subsequent studies[15].

Perhaps inevitably, this particular issue was not irretrievably buried by Morgan's study. For in 1968 its ghost appeared in the form of a letter to the Journal of the American Medical Association which suggested that all members of the 1948 Harvard rowing crew had since died "of various cardiac diseases"[16]; an assertion that was hotly denied by these oarsmen who reported shortly after, that they were all "alive and well"[17].

By default then, the last words on this topic must go to Sirs Alan Rock and Adolphe Abrahams. Introducing his own study of the longevity of Cambridge sportsmen, Sir Alan wrote[18]: "Too often when some well-known sportsman has died at an early age the Jeremiahs, shaking their heads mournfully, have connected two quite unrelated circumstances, much in the way that Morgagni recounted the case of the man 'who had been too much given to the exercise of tennis'. As this man died of a ruptured aortic aneurysm it seems likely that activities other than tennis were to blame". Sir Adolphe[19] had a similar story: "Some observers regard any circulatory disability as being attributable to exercise, and do not realize that exercise has revealed a latent organic defect in a heart hitherto regarded as sound. In an article in 1870 the Medical Officer to Rugby School, deprecating running on the account of the strain thrown on the circulation, noted that 'the recent death of England's greatest sprinter from aortic aneurysm is

significant'. So it is, but not exactly in the way he meant!"*

If it is accepted then that vigorous athleticism in youth does not reduce longevity, the second question arising from Morgan's study is whether the increased longevity of the University oarsmen resulted from their rowing. A detailed discussion of this question is relevant, because it introduces the concept of "selection versus protection". If this concept is not understood, it is not possible to critically analyze any studies postulating a direct relationship between physical activity and a particular health benefit such as increased longevity or a reduced incidence of coronary heart disease.

Morgan's study in fact allows no conclusions regarding the relationship between exercise and subsequent longevity. For the important reason that the two compared groups did not differ solely in whether or not they were physically-active. A number of "selective" factors were operative which would have favoured the longevity of the students even before they took to the water. Thus the oarsmen, because they were University students, would be expected to outlive Dr. Farr's "average" Englishman because as University students they enjoy greater longevity than the average population[20]. In the modern day this difference would be amplified because, due to their high social class, the University students would be expected to have a markedly reduced incidence of coronary heart disease[21,22]. Furthermore, the strenuous training of the oarsmen would probably have excluded any with health problems of sufficient gravity to impair their longevity,whilst the rowing coach would probably have

*Footnote: Lest these jokes be lost on those readers without medical backgrounds, I would point out that in the last century, syphilitic infection of the aorta was the most likely factor predisposing to fatal aortic rupture.

selected out the overweight and the heavy smokers - factors independently associated with reduced longevity[23,24] - even before their training had commenced. Thus the University oarsmen had been selected, in part because they were "healthy" and must have been in better than average health even before they started rowing. As Prout has written in a more recent study of oarsmen's longevity[25] - "obviously the oarsmen were in good condition to begin with, otherwise they would not have been chosen".

In contrast, Morgan's control group obtained from Dr. Farr's "English Life Tables", included the all-and-sundry Englishmen: the proverbially sick, the lame and the physically lazy. Therefore the 2-year longevity edge achieved by the oarsmen cannot be ascribed solely to their rowing because this was only one of the many factors differing between the groups.

Subsequent studies into the relationship between exercise and longevity have tried to overcome this problem of "selection" by comparing University (College) athletes with their classmates[15,26,27]. The over-riding conclusion from these studies has been that participation in University sports before World War II had little, if any, effect on the longevity or morbidity of the sportsmen. One must appreciate however that these studies do not address the question of whether life-long physical activity influences longevity, because in all these studies the exercising habits of the study groups were assessed only whilst they were students. No allowance was made for their activity patterns once they had left University.

2.2 EXERCISE AND CORONARY ARTERY DISEASE.

A. Epidemiological studies.

With the emergence of coronary heart disease as the epidemic disease of 20th century industrialized society, scientific interest in the potential therapeutic role of exercise has centered increasingly on its role in the prevention of this disease. The first major study evaluating whether physical activity (at work) influenced the mortality rate from coronary heart disease was reported by Morris and his colleagues[28]. Their essential finding was that when compared to their more sedentary colleagues, persons involved in physically-active occupations had reduced incidences of, and mortality rates from coronary heart disease, the latter due to a lower incidence of sudden and early (within 3 months) coronary deaths. Thus London bus conductors and postmen had less coronary heart disease than did respectively, bus drivers and sedentary employees of the Post Office. Likewise, in a review of deaths in London and the Home Counties, in each social class, workers in the more physically-demanding jobs had lower coronary heart disease rates, and were less likely to die during first or subsequent heart attacks than were those involved in occupations requiring little physical effort.

On the basis of these findings, these authors hypothesized that "men in physically-active jobs have a lower incidence of coronary heart disease in middle age than men in physically-inactive jobs. More important, the disease is not so severe in physically-active workers, tending to present first in them as angina pectoris and other relatively benign forms, and to have a smaller early case-fatality and a lower early mortality rate". In discussing their findings, these authors pointed out that the observed association between physical activity and reduced mortality rates from coronary heart disease, might be due to confounding variables other than physical activity. Thus they conceded that inherent constitutional differences

or psychological factors in the work environment might have been operative. As an example of this latter possibility, it was found that general practitioners had higher rates of coronary heart disease than did other groups of medical doctors.

Three years later, this group of researchers specifically studied whether constitutional factors influenced the choice of occupation. If such a relationship existed, their previous findings might indeed be spurious, explicable on the basis that constitutional factors determine both heart disease risk and choice of occupation.

By checking the trouser and jacket sizes of the uniforms issued to all employees of the London Transport Board, Morris, Heady and Raffle[29] showed that the girth sizes of the bus drivers were larger at all ages (25-59 years) than those of the bus conductors. As this difference was apparent even at the youngest ages, the conclusion must be that when they joined the London Transport system, the bus drivers had larger waists thán did the bus conductors. The bus conductors and the bus drivers were therefore of different builds, even before they commenced employment.

More recently, Oliver[30] has studied the body build and serum lipids of a group of young men selected by the London Transport Board for training as either bus drivers or bus conductors. He found that the trainee drivers were on average, taller by 2,6 cm, heavier by 3,3 kg, approximately 1 cm broader across the shoulders, and between 2 - 3 cm bigger round the chest and waists than were trainee conductors. Trainee drivers also had greater sub-cutaneous fat and marginally higher serum lipid values than did trainee conductors. Oliver concluded that "it is apparent that British men with certain physical characteristics choose or are chosen to become drivers as opposed to conductors" and that their study "supports the view that inherited characteristics, one of which may be susceptibility to heart disease, may predispose to a particular occupation". Despite this conclusion, it must be

emphasized that the exact extent to which these constitutional differences would effect the relative rates of coronary heart disease in either group, is not known.

Since publication of these early reports a large number of similar studies have been reported, the majority of which show a reduced incidence of coronary heart disease in the physically-active groups. These studies have been extensively reviewed and criticized[31,32] with particular attention being paid to the confounding variables present in each study. Of these studies, 3 most nearly exclude the presence of such variables in either of the study groups.

In 1973, Morris, Chase, Adam et al[33] reported that in a group of 16 882 male executive-grade Civil Servants, the relative risk of developing coronary heart disease in those who reported vigorous activity in leisure-time was about 1/3rd that of Civil Servants who did not report such activity. Vigorous leisure-time activity was also associated with a significantly reduced incidence of both rapidly-fatal and other forms of first heart attack, whereas the relative risk of heart attack in the most physically-active Civil Servants (those reporting "much" vigorous activity) was even lower than it was in those Civil Servants reporting vigorous activity. This population of Civil Servants constituted middle-class, white collar workers who were "very stable with much evidence of social responsibility and upward social mobility". When specific comparisons were made between those who did, and those who did not report vigorous leisure-time physical activity, it was found that both groups were highly comparable in terms of family compositions and worldly possessions, in heights and weights, in smoking incidences, and in those resident in towns supplied with hard and soft water. Whether inter-group personality differences existed was not determined. It is of interest that in this study, only <u>vigorous</u> and not <u>total</u> leisure-time physical activity was associated with a reduced heart disease mortality.

In a subsequent study, these workers investigated a subsample constituting 509 of the original 16 882 male Civil Servants[34]. They found that the group reporting vigorous leisure-time activity had significantly fewer electrocardiographic abnormalities compatible with myocardial ischaemia. This difference could not be explained on the basis of differences in either smoking prevalence or levels of either blood pressure or total serum cholesterol. Furthermore, although the electrocardiographic abnormalities in both groups increased with increasing blood pressure levels, in that group of men with the higher blood pressures, those reporting vigorous physical activity had fewer electrocardiographic abnormalities. Thus, the lack of vigorous leisure-time activity was a risk factor for ischaemic electrocardiographic changes, and it acted independently of 3 other major risk factors - smoking, hypertension and serum cholesterol levels. Furthermore, in the presence of established hypertension, vigorous leisure-time physical activity "protected" against ischaemic electrocardiographic changes.

More recently this group has reported a follow-up study of coronary mortality in a 20% subsample of their original study population[35]. In this subgroup of 3591 Civil Servants, there had been a total of 268 deaths by the end of 1977. Overall rates of coronary heart disease mortality and of mortality during the first or subsequent heart attacks, were again significantly lower in those reporting vigorous leisure-time activity. Although the incidence of cigarette smoking was slightly less in the vigorously-active group, members of this group had less coronary mortality whether or not they smoked cigarettes. Thus, as in the previous study, even in the face of an established coronary risk factor, vigorous activity "protected" against a manifestation of coronary heart disease. "Protection" was also shown to be specific for coronary heart disease, because there was no difference in mortality rates from cancer and other chronic fatal illnesses, between

those reporting, and those not reporting vigorous leisure-time activity.

Paffenbarger and his colleagues have also studied coronary mortality rates in groups differing in the amount of habitual physical activity performed either at work[36-39] or in leisure-time[40]. In their first study[36], they reported that in a cohort of 6 351 San Francisco Bay Area longshoremen, overall coronary mortality rates were significantly lower in those performing work which required bursts of high energy output. As in their subsequent studies[37,38] this "protection" was greatest against rapidly-fatal heart attack but even after a first heart attack, high energy output at work reduced subsequent mortality.

In their next study, Paffenbarger, Hale, Brand and Hyde[37] reported a 22 year follow-up of coronary risk factors in a subsample of 3686 longshoremen. They showed that the level of job activity, heavy smoking, high blood pressure and a history of heart disease were all significant risk factors for fatal heart attack. In addition, the risk attributable to low energy expenditure at work was greater than that associated with either cigarette smoking or raised blood pressure. As in the study by Chave, Morris, Moss and Semmence[35], the "protective" effect of high energy output at work was specific to cardiovascular mortality, there being no difference in mortality rates from other fatal chronic diseases, between heavy- and light-working longshoremen.

Paffenbarger[38] has considered whether these differences in coronary mortality rates could be explained on the basis of the initial selection of persons at low risk from coronary heart disease to jobs entailing high energy output. Convention in that industry precludes this however, because in their first five years of employment, all longshoremen must work in the group expending the most energy. Furthermore, the incidence of coronary risk factors was not greatly different between longshoremen involved in work requiring either high- or low-energy output. Thus blood cholesterol levels, systolic and diastolic blood pressures and the respective incidence(s) of

both abnormal glucose tolerance tests and diagnosed heart disease, were not different between groups. Body weight/height ratios and the incidence of cigarette smoking were however slightly less in the high energy output group; the latter difference Paffenbarger ascribes to the rule prohibiting high energy output workers, most of whom are cargo handlers, from smoking on the job. However, data analysis revealed that the small inter-group differences in these 2 risk factors could not explain their large differences in mortality rates. Furthermore, in the presence of 3 established risk factors - cigarette smoking, systolic hypertension or diagnozed heart disease - the high energy output workers had significantly lower incidences of fatal heart attacks.

Paffenbarger[38] concludes that these data "repeatedly point to a reduced work energy output level as equally as influential in increasing risk of fatal heart attack as such commonly recognized characteristics as cigarette smoking, prior heart disease and higher blood pressure..... reduced work energy output is more influential than obesity, abnormal glucose metabolism or higher blood cholesterol. This holds true after all adjustments and allowances have been made for the other personal characteristics".

In their most recent longshoremen study, Brand, Paffenbarger, Sholtz and Kampert[39] showed that statistical adjustments for age, race, cigarette smoking, systolic blood pressure, electrocardiographic abnormalities, glucose intolerance and body mass index failed to alter the correlation between the level of work activity and fatal heart attack rates, showing that work activity had a protective effect not explained through an indirect association with one or more of these other factors. Thus they conclude that the inverse relationship between work energy output and fatal heart attack rate is either directly related, or it is an indirect effect acting through modification of some other risk factor(s)

not accounted for in their analysis.

To assess whether leisure-time activity has a similar protective effect, Paffenbarger, Wing and Hyde[40] determined the risk of first heart attack in those 16 936 male alumni who had entered Harvard between the years 1916 and 1950. They found that alumni who expended more than 2000 Kcal per week in leisure-time physical activity had a significantly reduced incidence of first heart attack, although, in contrast to the longshoremen studies and the studies of the British Civil Servants, the incidence of fatal first heart attack was not significantly influenced by the level of leisure-time activity.

This "protective" influence against non-fatal first heart attack was found in all subgroups of active and inactive alumni, compared for their smoking incidence, body weight index, height, age, history of parental heart attack, systolic and diastolic blood pressures, and whether or not they had participated in sport whilst at Harvard. Of particular interest was the finding that only physical activity maintained during the period after graduation protected Harvard alumni against first heart attack; student athletes who became sedentary alumni received no protection from first attack, whereas physically-inactive students who subsequently became active after graduation received protection equal to that of physically-active alumni who had also participated in sports at Harvard. This finding argues strongly against the selection to life-long physical activity, of genetically-gifted athletes who are already at low risk of heart attack - the concept of "the fit shall increase their fitness"[33]. In the words of these authors: "if it is postulated that varsity sports participation reflects at least in part, a selective attribute of personal health (cardiovascular fitness), the present findings show that such selection alone is insufficient to explain lower heart attack risk in later

adult life".

The final argument preventing the acceptance of these studies[33-40] as scientific proof that physical activity reduces the incidence and mortality rates from coronary heart disease, centres on one aspect - namely the personal and psychological factors that determine work selection or choice of leisure-time physical activity. Such argument[41-43] holds that people who choose to become athletes or to work at physically-exacting tasks differ in some way, if not constitutionally then psychologically, from those who select sedentary occupations or leisure-time inactivity. It is postulated that these same unknown factors then shape, in some way, subsequent events.

The conclusion from these studies is that absolute, irrefutable epidemiological evidence showing that regular physical activity prevents or reduces coronary heart disease incidence or mortality, is not yet available and will only come from controlled randomized intervention studies which exclude all possible "selective" factors. The situation has been very succinctly summarized by Pollock, who has stated - "The evidence is on the fence. Which way you interpret it depends on how important you think exercise is".[44]

However the additional or alternate possibility that exercise training specifically protects against sudden coronary death, should not be overlooked. Thus, in the longshoreman studies reported by Paffenbarger and his colleagues[37-39], high energy output at work provided greater protection against sudden death than against death occurring 6 hours or later after the onset of symptoms. Similarly, in the British Civil Servants studies[33,35], not only were overall coronary mortality rates lower in those reporting, than in those not reporting, vigorous leisure-time activity, but sudden death mortality and mortality from first and subsequent heart

attacks was also significantly lower; findings compatible with a specific training-related protection against rapidly fatal heart attack. These findings have led Paffenbarger[45] to postulate that part of the myocardial adaptation to training may be a "stabilization of cardiac rhythm, and perhaps a reduced risk of suffering that chain of events proceeding from ectopic ventricular activity to fibrillation and death". This postulate provides the rationale for those studies undertaken in Chapter 4.

B. Experimental studies.

In view of the enormous difficulties posed in mounting major epidemiological studies exploring the relationship between exercise and coronary heart disease, a simpler experimental approach to this question, is to study the effects of exercise training on either the extent of, or the rate of progression of coronary atherosclerosis in either animals with experimentally-induced atherosclerosis or in humans with the naturally-occurring disease.

Some animal studies have indeed shown that exercise training can reduce or prevent the development of cholesterol-induced atherosclerosis in rabbits[46,47], pigs[48], cockerels[49,50], chickens[51] and monkeys[52]. It should however be noted that other studies in rabbits[53] and chickens[54] failed to show a protective effect of chronic exercise on cholesterol-induced atherosclerosis, whereas McAllister, Bertsch, Jacobson and D'Alessio[55] even reported that dietary-induced atherosclerosis was actually increased in exercising dogs.

In human autopsy studies, although no relationship has been found between occupational physical activity levels and the extent of coronary atherosclerosis[56-58], less occlusive coronary artery disease[56] and less ischaemic myocardial fibrosis[56] has been observed in the occupationally

physically-active groups, suggesting that exercise might have a protective effect either against coronary artery occlusion, or against the myocardial response to acute ischaemia.

Evidence for this latter hypothesis from the work of Rissanen[59] who found, in a study of persons dying from coronary heart disease, that the physically-active were less likely to die either suddenly and unexpectedly without a previous history of clinical ischaemic heart disease, or to die as a consequence of single or double vessel disease. He suggested that a different myocardial response to occlusive coronary artery disease may exist in physically-active men, and that this would explain why overall, active men were under-represented in his separate series of sudden cardiac deaths.

In patients with known coronary artery disease preliminary studies suggest that although exercise training may not cause regression of established coronary atherosclerosis measured angiographically[60-67], it may reduce the rates of atherosclerotic progression in the femoral[68] and coronary arteries[69]. Additional studies are clearly indicated.

C. The "Bassler hypothesis".

In 1972, a letter written by an American pathologist Dr. Tom Bassler, appeared in the Lancet proclaiming that "a search of the literature by the American Medical Joggers Association failed to document a single death due to coronary atherosclerosis amongst marathon finishers"[70]. In the 5 years following this initial publication, a further 24 letters appeared in a variety of international medical journals, concluding that this absence of documented cases of "fatal coronary atherosclerosis"*

*Footnote: The meaning of this term has never been exactly defined.

in marathon runners indicated that marathon running provided immunity against coronary atherosclerosis[70-79], ischaemic heart disease[80,81], fatal myocardial infarction[82-84], "loafer's heart"[85] and coronary heart disease[86-95].

The "Bassler hypothesis" as it subsequently became known[96,97], raises a number of interesting possibilities. First, it is in line with those experimental studies reviewed in the previous section, showing that at least in the animal model, exercise training can prevent experimentally-induced atherosclerosis. Second,it is compatible with the concept that only exercise above a certain "threshold" intensity protects against coronary artery disease[33,36,40]: the implication of the Bassler hypothesis being that marathon running exceeds this threshold by so much, that it provides absolute,not partial,protection from this disease. Third, the alternate possibility is that if marathon runners truly are immune to death from coronary artery disease but are not absolutely protected from coronary atherosclerosis, then one possibility (ignoring for the moment the obvious possibility of "selective" factors) is that exercise training increases myocardial resistance to those factors promoting life-threatening ventricular arrhythmias as discussed in the previous section.

My studies which have helped differentiate between these possibilities and have disentangled the "cardiomythology" of marathon running[41], are described in Chapter 3.

2.3 MYOCARDIAL ADAPTATIONS TO EXERCISE TRAINING.

The models used in the study of the effects of exercise training on the heart can be broadly grouped into those in which the heart or a part of it is isolated and studied *in vitro*, or those in which the heart is studied whilst it remains *in vivo* in its anatomically correct position (Table 2.1).

In reviewing these studies, I have not tried to integrate all the results into a unifying concept of how the heart adapts to exercise training. Where some degree of consensus exists I have alluded to this, but in most instances due to the different experimental models and the conflicting results, this has not been possible. Rather have I tried to bring together all the relevant literature in a logical manner, so that the discrepancies as well as the consistencies will be apparent.

TABLE 2.1

EXPERIMENTAL MODELS USED TO STUDY MYOCARDIAL ADAPTATIONS TO TRAINING:

ISOLATED TISSUE STUDIED IN VITRO

Structure studied	Typical parameters measured
Whole heart	Heart weight, biochemistry, extent of coronary vessels
Tissue slices	Histology and ultrastructure
Mitochondria	Mitochondrial function
Sarcoplasmic reticulum	Calcium uptake
Papillary muscle	Contractile function
Isolated rat atrium	Intrinsic heart rate
Isolated rat heart	Function and metabolism

HEART STUDIED IN VIVO

Non-invasive studies.

Chest radiography	Heart size
Echocardiography	Heart size and function
Respiratory gas analysis	Cardiac output during exercise

Invasive studies.

Chronically-instrumented animal	Left ventricular function during exercise
Coronary sinus intubation	Metabolism and coronary flow
Coronary angiography and left ventriculography.	Coronary anatomy and heart function
Radionuclide cine-angiography	Heart function during exercise

A. Structural and ultrastructural adaptations.

Heart weight.

As reviewed by Grande and Taylor[98], the evidence is that the weight of hearts from wild animals is greater than that of their domestic relatives. Thus wild ducks, greyhounds and hares have heavier hearts than do respectively, tame ducks, other dogs and rabbits. Thoroughbred racehorses also have larger hearts than do ordinary hacks and the largest hearts have been found in the most successful racehorses[99]. A similar finding has been reported in greyhounds; the dog with the most successful track record - "Victorious Red" - also had the largest heart[100].

The assumption has been made that the greater heart weights of these wild animals and of animals bred for racing, result from their greater physical activity. However, such cross-sectional studies do not exclude the possibility that inherited characteristics explain these differences. Hermann[100] considered this when he found that even young, untrained greyhounds had greater heart weight to body weight ratios than did other dogs. He suggested that "the greyhound, by virtue of generations of strenuous exercise of coursing, or as a result of selection, is endowed with a proportionately large heart at birth and that this heart responds to schooling and training by hypertrophying to an unusual degree".

For obvious reasons, there are few reports of the heart weight of athletes measured whilst they were in strenuous training. As cited by Grande and Taylor[98], Reindell and his colleagues collected heart weight data from the autopsies of 40 German athletes. The majority of these hearts weighed less than 450 gm with only 3 weighing more than 500 gm. The heaviest heart, that of a famous mountain climber who died in cardiac failure, weighed 840 gm - a value that could almost certainly have resulted only from a pathological process. At his death, the heart of

Clarence de Mar, the famous American marathon runner, weighed 340 gm[101]. As de Mar died from carcinoma of the rectum one year after his last running race[102], it is likely that his heart had undergone some terminal atrophy. These data, scanty as they are, suggest that in man, any training-induced increase in heart weight is likely to be quite small.

Longitudinal studies of the effects of physical training on heart weight in laboratory animals have been reviewed by both Grande and Taylor[98] and by Scheuer and Tipton[103]. The latter authors make three critical observations:

1) that an increase in the heart weight to body weight ratio after training is not an acceptable criterion for ventricular hypertrophy because, especially in young male rats, regular exercise retards the rate at which body weight increases during the growing period. Thus the heart weight to body weight ratio will be increased in growing, trained, male animals whether or not true ventricular hypertrophy has also occurred;
2) that an increase in absolute heart weight is <u>not</u> a universal finding in all studies in which laboratory animals have been trained either by swimming or by running; and
3) that certain myocardial adaptations to training may be present even in the absence of cardiac hypertrophy.

These authors conclude that the final resolution of this question will come from histological studies that define the normal myocardial cell size relative to the sex, weight and nutritional status of the animal, and the effects of exercise thereon.

Dowell, Tipton and Tomanek[104] have been the first to undertake such a study. They found that whereas both aortic constriction and desoxycorticosterone acetate injections increased both left ventricular

weights and myocardial fibre sizes indicating true left ventricular hypertrophy, 80 to 90 days of treadmill running-training had no such effect. As expected, the body weight of the trained animals was less than that of the controls. These authors concluded that treadmill-training in male rats produces relative left ventricular enlargements by allowing myocardial masses to increase at normal rates whilst simultaneously reducing the overall growth rates of the animals. Under these conditions, normal rates of heart growth would cause relative cardiac enlargements which would therefore be achieved without absolute increases in either myocardial fibre sizes or rates of protein synthesis.

In a somewhat contradictory study Hickson, Hammons and Holloszy[105] found that rats exposed to a constant (rather than a gradually-increasing) swimming workload developed absolute increases in heart weights within 14 days training. The increased heart weights were associated with parallel increases in total protein contents and significantly increased total RNA contents. These changes rapidly regressed when training ceased.

Studies relevant to the question of exercise-induced cardiac hypertrophy in humans are reviewed in Section 2.3E - Echocardiographic evaluation of cardiac structural adaptations to exercise training.

Myocardial fibre size, fibre count and sarcoplasmic mass.

As discussed by Scheuer and Tipton[103], an exercise-induced increase in myocardial fibre size would provide strong evidence that exercise training does indeed produce true cardiac hypertrophy.

In rats trained with either treadmill-running[104,106] or swimming[107], no change in myocardial fibre size has been found. On the other hand, increased myocardial fibre diameters have been reported in running-trained guinea-pigs[108] and dogs[109]. Bloor, Pasyk and Leon[110],

and Leon and Bloor[111] reported that hearts from young, male, swimming-trained rats had increased absolute heart weights due to both a 26% increase in myocardial fibre counts and increased sarcoplasmic mass per fibre. The increase in sarcoplasmic mass was found only in rats who had swum for 1 hour a day, 5 days a week. With de-training these adaptations regressed, but the myocardial hyperplasia could be maintained by a single hour-long exercise session every week. An age effect for these adaptations was also apparent, because in older rats, training caused decreased heart weights due to decreases in both myocardial fibre counts and sarcoplasmic masses.

Myocardial mitochondrial mass, volume, number and size distributions.

Using precise morphometric analysis, Paniagua, Vasquez and Lopez-Moratalla[107] found no increase in either mitochondrial volume or mass in hearts from rats that had swum for up to 2 hours a day for 6 weeks. On the other hand, Arcos, Sohal, Sun et al[112] reported increased myocardial mitochondrial masses estimated to be due to increased numbers of mitochondria, in hearts of rats who had swum for a total of between 140 and 180 hours. However hearts from rats that had swum for longer total periods (361 to 490 hours) failed to show these increases. In a study reviewed by Wollenberger[113], Bozner and Meerson reported that myocardial mitochondrial to myofibrillar volume ratios increased rapidly after 4 training sessions in which rats swam 6 hours daily, but were returning towards control values after 40 training sessions. In Wollenberger's own studies, rats that had swum for between 60-90 minutes daily for 4 months, had moderate myocardial hypertrophy and slightly increased mitochondrial to myofibrillar volume ratios. Wollenberger concludes that at the cellular

level, the myocardial response to training is not a uniform process, as the mitochondria apparently respond earlier to increased workloads than do the myofibrils.

Using the same technique as that of Arcos and his colleagues[112], Aldinger and Sohal[114] "thought" they observed increased numbers of mitochondria and increased mitochondrial to myofibril ratios in hearts from swimming-trained rats. These authors also noted degenerative changes in many mitochondria, changes which were not present in the mitochondria of similarly-trained rats who had received digitoxin during the training programme. (The significance of these "degenerative" mitochondrial changes found in rats exercised for prolonged periods has subsequently been studied by Tomanek and Bannister[115], and Gale[116], all of whom found that such changes are almost certainly post-mortem staining artefacts and can be prevented either by rapid fixation of the heart, or by fixation in a cacodylate buffered glutaraldehyde solution. Other workers have not found degenerative mitochondrial changes after acute, exhaustive exercise [117,118].)

Edington and his colleagues[119-121] have studied the effects of training on mitochondrial size distributions. They found that although training has no effect on total mitochondrial protein contents (section 2.3B), trained hearts had greater proportions of small mitochondria, particularly in the myofibrillar region[120], an adaptation which these authors considered to be of potential physiological significance because it would cause increased mitochondrial surface to volume ratios. In a subsequent study[121], these authors found that training prevented an age-related increase in the percentage of large mitochondria found at the cell borders and in the myofibrillar regions of the heart. They concluded that training maintained myocardial mitochondrial distributions that more closely resembled or

exceeded those found in young animals.

A final topic that has been studied is whether a single exercise bout increases either myocardial mitochondrial masses or volumes. Laguens and his colleagues concluded that a single bout of exercise in dogs[122] and rats[123] could increase mitochondrial masses, a conclusion which is in keeping with the finding of increased protein incorporation into mitochondria of rats swum to exhaustion in a single session[124]. Terjung, Klinkerfuss, Baldwin et al[117] have however questioned these conclusions because they found that a single bout of exhaustive exercise failed to increase either myocardial mitochondrial protein contents, or myocardial metabolic capacities,measured as the maximum rates of pyruvate oxidation,by either whole heart homogenates or by mitochondrial fractions.

When all the studies reported in this section are reviewed, it becomes difficult to decide whether or not exercise training definitely alters myocardial mitochondrial masses, numbers or volumes. Biochemical studies that supply further information on this topic are reviewed in sections 2.3B and 2.3C.

Myocardial intercalated discs.

Sohal, Sun, Colcolough and Burch[125] and Aldinger and Sohal[114] studied the myocardial intercalated discs of swimming-trained rats. They reported that both the convolutions in the intercalated discs and the intercellular gaps appeared to be increased in hearts from trained animals. They suggested that if these electronmicroscopic appearances reflected true structural hypertrophy and if ionic transfer occurs in the region of the intercellular gaps, then there could be increased efficiencies of electrical excitation in hearts of trained animals.

Coronary blood vessels.

Four principal techniques have been used to study the effects of exercise training on coronary blood vessels:

1) the microscopic determination of coronary artery sizes, capillary concentrations and the myocardial capillary/fibre ratios;
2) the incorporation of radioactively labelled ^{3}H-thymidine into capillary nuclei;
3) corrosion cast techniques in which a resistant polymer solution such as vinyl acetate in acetone is injected in vivo into the aorta of the experimental animal, whose heart is subsequently excised. The polymer is allowed to set before the heart is placed in a potassium hydroxide solution which digests the myocardial tissues leaving the intact cast of the coronary vessels; and
4) coronary angiography.

It is important to appreciate that these studies indicate the anatomical extent of the coronary blood vessels but provide no information about possible training-induced changes in either rates or distributions of coronary blood flow. Studies relevant to these latter questions are reviewed in section 2.3F.

1) Microscopic studies.

The earliest microscopic studies reporting the effects of exercise training on the myocardial vasculature are in conflict, because although Petren and his colleagues, and Thorner found increased capillary concentrations in hearts of running-trained guinea-pigs[126,127] and dogs[109] respectively, both Frank[128] and Hakkila[108] reported decreased myocardial

capillary concentrations in running-trained guinea-pigs. Furthermore the capillary counts in Hakkila's study were much greater than those reported by Petren's group and were verified by 3 different counting techniques.

Leon and Bloor[129] reported that the capillary/fibre ratios, the left and right coronary artery sizes (0,5 mm from their respective origins) and the cross-sectional areas of the extra-coronary collateral arteries (these are vessels that result from anastomoses between the internal mammary or less commonly the subclavian arteries, with atrial branches of the coronary arteries) were all significantly increased in hearts from young male swimming-trained rats. Coronary artery diameters were however increased only in that group of rats that also developed myocardial hypertrophy - a finding that is consistent with the known correlation between coronary artery diameter and heart weight[130-132]. Subsequent studies[110,111,133, 134] confirmed these findings. In addition they showed that the increased capillary/fibre ratios in older exercising rats were due to a loss of myocardial fibres rather than to increased capillary concentrations.

These authors have also studied the extent to which reduced training loads maintained these adaptations. They found that 30 minutes' exercise twice-weekly was required to maintain the increased capillary concentrations whereas a 1-hour training session once a week maintained the enlargement of the extra-coronary arteries. Myocardial hypertrophy and the associated increased coronary artery diameters, was maintained only by 5 one-hour exercise sessions per week, but after 4 weeks' total inactivity these adaptations had completely regressed. The increased capillary/fibre ratios were still present after 4 weeks' detraining but after a further 6 weeks these too had regressed.

Bell and Rasmussen[135] and McElroy, Gissen and Fishbein[136] have also reported increased myocardial capillary/fibre ratios in hearts from swimming-trained rats. In addition, Bell and Rasmussen found that capillary/fibre ratios increased normally with age, and that this increase was enhanced by training either before or during puberty. In running-trained rats, Tomanek[106] and Welch, Manfredi and Edington[121] found increased myocardial capillary/fibre ratios but the magnitude of the increase (8,5%) in the former study was less than that reported in swimming-trained rats (43%)[129]. Tomanek also observed that the extent to which exercise influenced myocardial capillary concentrations decreased with age and was significant only for the rats that commenced training whilst still young.

Wyatt and Mitchell[137] performed serial ventricular septal biopsies in running-trained dogs and reported small but not statistically-significant increases in myocardial capillary densities and capillary perimeters with decreases in basement membrane thicknesses. With de-training these adaptations regressed. The authors noted that the 6% increase in myocardial capillary densities measured by them is comparable to values found in other adult animals, and that larger increases have been reported only when young animals have been studied (vide supra).

Ljungqvist and Unge[138] injected radio-opaque dye into the coronary arteries of swimming-trained and control rats immediately before death. The hearts were then removed and fixed in formalin, whereafter 4-5 mm transverse sections were cut through the ventricles and exposed on a photographic emulsion. These "micro-angiograms" were then studied with a special stereoscopic microscope, after which the tissue slices were re-cut, stained in the conventional way and analysed under the light microscope.

It was found that on the micro-angiograms, the capillary network appeared to be denser whereas when compared to hypertrophy produced by either aortic stenosis or renal hypertension, the light microscopic capillary architectural appearance in trained hearts was different because neither segmental filling defects nor spirallization of the vessels was noted. After 2 months de-training, these changes had not completely regressed.

The influence of maternal exercise during pregnancy on the capillary/fibre ratios in the hearts of their offspring has also been studied. Whereas Pařízková[139,140] reported that maternal exercise increased this parameter, Wilson and Gisolfi[141] failed to find this adaptation. These latter authors discussed some of the methodological differences that might explain these contrasting results.

Finally, it is of interest to note that the myocardial capillary/fibre ratios are greater in wild animals (hare or wild rat) than in their domesticated relatives (tame rabbit or rat)[142]. However, a genetic explanation for this cannot be excluded (section 2.3A).

2) The incorporation of radioactively labelled ^{3}H-thymidine into capillary nuclei.

In this technique developed by Ljungqvist and his colleagues[143-147] radioactively labelled ^{3}H-thymidine is injected intraperitoneally into swimming-trained and control rats one day before sacrifice. As thymidine is taken up selectively by DNA-synthesizing cells, the degree to which a particular tissue incorporates ^{3}H-thymidine may be taken as a measure of that tissue's proliferative activity.

After sacrifice, the hearts are fixed in formalin, and transverse ventricular sections placed on glass slides to which photographic film has been attached. Capillary cells that are actively dividing and have there-

fore taken up radioactive label will expose the photographic film and can therefore be counted.

These authors found highly significant increases in total labelling indices in the capillaries only of hearts hypertrophied by swimming. Other methods for producing left ventricular hypertrophy (renal hypertension and aortic stenosis) did not cause increased ^{3}H-thymidine incorporation into myocardial capillaries. The authors concluded that the formation of additional myocardial blood vessels occurred only in physiologic forms of myocardial hypertrophy, such as that produced by swimming training. Three additional observations were made by these authors:

(i) that after 2 months de-training, thymidine incorporation into the capillaries had returned to control levels;

(ii) that hearts from rats with experimentally-produced aortic stenosis who had also undertaken the swimming training programme, did not show new capillary formation[144]; and

(iii) that the skeletal muscles of swimming-trained animals did not develop new capillaries.

Using the electron microscope, Mandache, Unge and Ljungqvist[146] attempted to locate the cellular basis for this training-induced new capillary formation. They noted increased numbers of mitotic figures in relation to the capillary walls. Growing capillaries appeared first as solid cords composed of pericyte-like cells surrounding endothelial-like cells. When these cords established contact with the original capillary lumen, they enlarged to form a continuation of that lumen. These indicators of new capillary growth were already present after 2 weeks training.

In a final study[147], these authors combined both the above techniques (electron microscopy and ^{3}H-thymidine labelling) and showed

that ^{3}H-thymidine was taken up mainly by endothelial cells, but labelled capillary pericytes were also observed. As previously shown, no such uptake was seen in hypertrophied hearts from animals with aortic stenosis. This study therefore confirms that the increased capillary ^{3}H-thymidine labelling seen in their previous studies was in fact due to endothelial cell replication.

3) Corosion cast techniques.

Using this technique Tepperman and Pearlman[148] prepared casts of the coronary vessels of running-trained rats and showed that cast weights were significantly increased in trained animals. Similarly, Stevenson, Feleki, Rechnitzer and Beaton[149] showed that both cast weights and cast weight/heart weight ratios were significantly increased in rats trained with either swimming or running. Surprisingly, the most intensive running or swimming programmes did not cause increased cast weights. Denenberg[150] also reported increased cast weight/heart weight ratios in running-trained adult rats exercised 3-5 times a week and in adolescent rats exercised 5 times a week.

This latter author discussed some of the technical problems associated with the corrosion cast technique. These include non-randomization of the "blind" infusions, the need for strict control of the pressure used to perfuse the polymer and the need to compare results obtained by this technique with parallel histological studies. A further criticism voiced by Wyatt and Mitchell[137] is that vinyl acetate perfuses only arterial vessels with a diameter greater than 40-50 μm and thus gives no information on the dimensions of the capillary bed. Thus changes in cast weights with training may simply indicate increased coronary artery sizes concomitant with increased heart weights[130-132] although

in 2 of the studies reviewed in this section, heart weights were not increased in trained animals[149,150]. The other study[148] fails to mention heart weights.

4) Coronary angiography.

Using magnified projections of the antero-posterior views obtained during coronary angiography, Wyatt and Mitchell[137] calculated that the resting cross-sectional areas of the circumflex coronary arteries were significantly increased in running-trained dogs. With de-training circumflex coronary artery areas returned to normal values.

B. Myocardial biochemical changes with exercise training.

Myocardial levels of ATP, ADP, AMP, PCr, adenosine, inosine, hypoxanthine, phosphate, creatine and creatinine.

Three studies[151-153] have not found any change in either myocardial ATP or PCr levels after training. Others have reported significantly decreased ATP[154,155] and significantly increased PCr levels[156] in hearts from trained rats. Similarly, ADP and AMP levels have been reportedly increased[154,155] and unchanged[151] following swimming training in rats. Myocardial levels of adenosine[155], inosine[155], hypoxanthine[155], creatine[156], creatinine[156] and inorganic phosphate[151] were unchanged in hearts from trained rats.

Myocardial glycogen, lactate, cholesterol and triglyceride levels.

As recently reviewed by Segel[157], studies to determine whether training alters myocardial glycogen levels have produced conflicting results, which may possibly be explained on the basis of a training-induced

increase in myocardial glycogen synthetic capacity during fasting. This would explain why 4 studies[151,158-160] have all found significantly higher myocardial glycogen levels in hearts from fasted, trained rats than from similarly-fasted, control rats. When animals were killed without a period or prior fasting, Chelley, Code and Visscher[158] reported no difference in myocardial glycogen levels in hearts from trained and control animals. Similarly, Scheuer, Kapner, Stringfellow et al[151] observed that a 24-hour period of fasting prior to sacrifice "yielded more consistent differences in glycogen values between sedentary and conditioned animals". Other investigators who have studied fed animals have not reported training-induced differences in myocardial glycogen levels[153,161-164].

Myocardial lactate levels were not different between trained and control animals, whereas myocardial triglyceride levels have been reportedly decreased[151,165] or unchanged by training[166]. Myocardial cholesterol levels were also unchanged in the latter study[166].

Myocardial glycolytic and related enzymes.

The activities of pyruvate kinase[162,167], hexokinase[168], lactate dehydrogenase[107,169-171] including the M subunit[162] of LDH, have all been reported to be increased in hearts from trained animals. Some authors did not find any change in myocardial LDH[172,173] or hexokinase activities[164]. The activity of phosphofructokinase, an important glycolytic regulator, has not been shown to increase in response to exercise training[162,168,174]. Myocardial aldolase activities have been reported to be increased[175] or unchanged[176] by the same authors. Myocardial glycerol-1-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and cytoplasmic creatine kinase activities were unchanged in hearts of swimming-trained rats, whereas cytoplasmic adenylkinase activities were increased[171].

Myocardial glycogenolytic and glycogen synthetic enzymes.

Segel and Mason[163] found no difference in myocardial synthase I (glucose 6-phosphate-independent activity), synthase D (glucose 6-phosphate-dependent-activity), I + D activities or synthase activation states $\left[I/(I+D)\right]$ measured in hearts from trained and control rats after a period of rest.

These authors also studied the effects of prior exercise on the extent of myocardial glycogen resynthesis in trained and control animals. They showed that whereas mild exercise caused synthase activation and glycogen supercompensation in hearts from the control group, only strenuous exercise caused synthase activation and glycogen supercompensation in the trained group. They concluded that exercise training alters the pattern of glycogen supercompensation following a given bout of exercise.

Clearly, the conflicting results regarding the effects of exercise training on myocardial glycogen contents could be resolved if studies similar to that of Segel and Mason[163] were used to compare glycogen metabolism in hearts from trained and control animals during fasting.

Myocardial enzymes associated with aerobic metabolism.

The majority of studies have shown that exercise training does not increase the enzymes typical of aerobic metabolism in heart muscle. Thus no change has been found in succinate dehydrogenase[113,153,173,177-181], citrate synthase[174,181], cytochrome oxidase[107,161,176,179-182], mitochondrial malate dehydrogenase[107,171,181], succinate-cytochrome C reductase[176], glutamate oxalate transaminase[171], isocitrate dehydrogenase[171], glutamate dehydrogenase[171], 3 hydroxy acyl CoA dehydrogenase[171], mitochondrial adenyl kinase[171] or mitochondrial creatine kinase[171].

Mitochondrial ATPase activities[107,152,182] and the levels of cytochrome C have also been found not to be increased by training[104-106, 179-181,183].

In contrast to these studies, some authors found increased activities of succinate dehydrogenase[168,184,185], mitochondrial glycerol-P dehydrogenase[185], malate dehydrogenase[168], citrate synthase[168] and 3 hydroxy acyl CoA dehydrogenase[153]. It should be noted that in the study by Harri and Valtola[168], cold acclimatization ($4^{o}C$) also increased the activities of myocardial succinate dehydrogenase, malate dehydrogenase and citrate synthase. Furthermore, in that study, the water temperature in which the rats swam was $30^{o}C$, a temperature which is considered sub-optimal[186]. It is possible that the water temperature used in that study influenced the myocardial respiratory enzyme adaptations.

In summary, the weight of evidence is that the activities of the myocardial enzymes associated with aerobic metabolism do not increase in response to exercise training. From this it has been concluded that the untrained heart has a sufficiently great aerobic reserve capacity to be able to cope with the additional stress of exercise without the need for additional mitochondrial enzyme synthesis. The final resolution of this question is most likely to come from metabolic studies performed in the intact animal during maximum exercise.

Myocardial cytoplasmic and mitochondrial protein contents, and myocardial RNA, DNA and hydroxyproline levels.

In the study reported by Walpurger and Anger[171], training caused a 15% decrease in myocardial cytoplasmic protein contents. The majority of workers have not found any change in either myocardial protein contents[104,105,168,187-190] or mitochondrial protein concentrations[107,119, 178,184], or in myocardial DNA[6,183,191,194], RNA[104,119,178,188,191],

hydroxyproline[104,188,192,193] or collagen levels[194].

In contrast, Penpargkul, Schwartz and Scheuer[182] reported a 52% increase in absolute mitochondrial protein contents of hearts from swimming-trained rats. They suggest that the technique whereby mitochondria are isolated may cause a significant number of mitochondria to be lost so that the "mitochondrial yield", used by some authors as an index of in vivo myocardial mitochondrial content may be an underestimation. Hickson, Hammons and Holloszy[105] reported that myocardial RNA concentrations of rats exposed to a constant swimming programme (section 2.3A) increased rapidly in the first few days of training, being significantly elevated in the first 3 training days. Thereafter these values fell but after 28 training sessions, total RNA contents still remained significantly higher than control values. Both DNA and hydroxyproline concentrations fell progressively during training but total ventricular DNA and hydroxyproline contents were nevertheless significantly increased by training. Total cytochrome C and total protein contents, but not their concentrations, also increased with training. During detraining, with the exception of the increased total hydroxyproline content which remained elevated, all other changes reverted to normal.

Beecher, Puente and Dohm[195] have reported that endurance training increased the myocardial levels of only the basic amino-acids, arginine and lysine.

Other myocardial enzymes.

Myocardial lipoprotein lipase is not increased by training[196]. Sarcolemmal Ca^{++}- and Mg^{++}-ATPase activities were significantly increased in swimming-trained hearts[197], whereas Na^{+}-K^{+} ATPase activities were

unaltered by training[197,198]. Sarcoplasmic reticulum Ca^{++}- and Mg^{++}-activated ATPases were also unaltered by training[199-201].

Actomyosin and myosin ATPase activities.

Studies of the effects of training on actomyosin and myosin ATPase activities have produced conflicting results which can probably be explained on the basis of the type of exercise studied. Thus swimming training has consistently been shown to increase myocardial[202], myosin[193,203-206] and actomyosin ATPase[193,206-210] activities. These swimming-induced changes in myosin ATPase activities have been explained on the basis of altered reactivity of the sulphydryl group of myosin[211]. Medugorac[206] found an increased light chain 1 component (slow moving) of myosin associated with increased myosin and actomyosin ATPase activities.

In contrast, with the exception of one study showing small changes [201], endurance running training does not alter actomyosin ATPase[212-216], myofibrillar ATPase[174,215-217] or myosin ATPase activities[213,218,219]. Nor does running-training induce changes in the subunits of cardiac myosin[213,219]. When rats undergo sprint training however, increased myocardial myofibrillar ATPase activities have been reported[174].

Pierce, Belcastro and Bonen[164] investigated changes in Mg^{++}-activated cardiac myofibrillar ATPase activities during the initial training period. They found increased enzyme activities after 3-5 training sessions but after 10 training sessions, myofibrillar ATPase activities in trained animals had returned to control values.

Myocardial electrolyte and water contents.

Myocardial potassium contents have been reportedly increased in hearts from both swimming-[198] and running-trained rats[220]. Swimming training did not alter myocardial levels of sodium, calcium, zinc and copper[198], nor mitochondrial calcium contents[182]. A number of studies have reported no change in myocardial water contents in trained hearts [180,189,190,195,198].

Myocardial levels of catecholamines, acetylcholine, and the activities of choline acetyltransferase, adenylcyclase and phosphodiesterase.

Catecholamines

De Schryver and his colleagues[221-223] have reported that running training decreased rat myocardial catecholamine levels but that these values had returned to control levels within a few days detraining. The greatest reduction in myocardial catecholamine levels occurred in those rats which had trained the most intensively. Furthermore, myocardial catecholamine levels in control animals which refused to run freely in the exercising drums but which chose to slide passively as the drums rotated, showed no reduction in catecholamine levels. Thus stimuli other than exercise, such as daily handling or exposure to the running drums could not explain the reduced myocardial catecholamine levels in the trained animals. Amsterdam, Choquet, Segel et al[224] and Wollenberger and his colleagues[225,226] have also reported lower myocardial catecholamine levels in swimming-trained rats.

In contrast, others have reported that running training did not reduce myocardial noradrenaline levels in either guinea-pigs[227] or rats[228,229],

whereas swimming training was associated with unchanged[230] and significantly increased[231] myocardial noradrenaline levels. A criticism of the study by Leon, Horst, Spirt et al[230] has been that hearts were frozen only some considerable time after removal. Thus prior to freezing, some noradrenaline may have been metabolized by the myocardial enzymes, monoamine oxidase and catechol-O-methyltransferase[157].

However, evaluation of tissue catecholamine levels gives little definitive information of their functional significance. For example, despite significantly higher resting myocardial noradrenaline levels, the rates of noradrenaline turnover measured both at rest and during exercise in hearts from swimming-trained rats were half those of untrained animals[231]. Furthermore, rates of urinary catecholamine excretion during exercise were significantly less in the trained animals whereas after very prolonged exercise (8 hours' swimming), myocardial noradrenaline levels were significantly reduced only in hearts from untrained animals[231].

Salzman, Hirsch, Hellerstein et al[232] studied the <u>in vitro</u> uptake of radioactively-labelled ^{3}H-epinephrine by myocardial homogenates from running-trained mice. They found that when expressed per milligram of tissue, ^{3}H-epinephrine uptakes were 26% lower in hearts from trained animals. However, when expressed relative to total ventricular weights this difference was lost, because the hearts of trained animals were correspondingly larger. They concluded that concurrent with the development of exercise-induced cardiac hypertrophy, there was dilution of cardiac epinephrine binding sites. The authors also reported a sex difference in their findings, because hearts from both trained and control female mice had, relative to left ventricular weights, 15% greater

epinephrine uptakes than did hearts from the corresponding male groups.

These findings in particular those of Ostman, Sjöstrand and Swedin[231] suggest that, regardless of its effect on total tissue catecholamine levels, prolonged physical training produces a functional myocardial adaptation characterized by reduced myocardial catecholamine activation both at rest and during exercise. This is a parallel to the training-induced reduction in overall sympathetic tone in humans[234-236].

Acetylcholine and choline acetyltransferase activity.

Both Herrlich, Raab and Gigee[237] and De Schryver and Mertens-Strythagen[238] found significantly increased acetylcholine levels, localized in the former study to the atria, in hearts from trained rats. Tipton, Mathes, Tcheng et al[229] also reported increased, though not statistically significant, acetylcholine concentrations in both atrial and ventricular tissue of trained rats. On the other hand, Stone[239] did not find any change in either acetylcholine or choline concentrations in right atrial tissue from running-trained dogs.

Increased activities of the acetylcholine synthetic enzyme choline acetyltransferase have been reported in atria, but not ventricles, of running-trained rats[240]. Acetylcholinesterase activities were unchanged in running-trained rats[241,242] and significantly decreased in running-trained dogs[239].

Adenylcyclase and phosphodiesterase activity.

Dohm, Pennington and Barakat[243] found that hearts from running-trained rats had reduced basal and epinephrine-stimulated adenylcyclase activities with unchanged myocardial phosphodiesterase activities. Fluoride-stimulated myocardial adenylcyclase activities were not different

between trained and control animals, indicating that the training-induced reduction in adenylcyclase activities were due either to alterations in the control properties of the enzyme or to the presence of endogenous effectors, rather than to changes in enzyme concentrations. They concluded that physical training alters the capacity of the myocardium to respond to hormonal stimulation.

In contrast, Wyatt, Chuck, Rabinowitz et al[244] found that control or fluoride-stimulated adenylcyclase activities were not different in swimming-trained and control rat hearts. In response to either isoproterenol or noradrenaline stimulation, the maximum increases in adenylcyclase activities were significantly greater in trained than in control hearts.

Additional work will be required to determine whether these conflicting results are due to the different training methods (swimming versus running) used by the 2 groups of investigators.

C. Myocardial metabolic adaptations to exercise training.

Five principal models - myocardial homogenates, isolated myocardial mitochondria, isolated sarcoplasmic reticulum, isolated perfused rat hearts and intact animals - have been used to study the effects of exercise training on myocardial metabolism.

Myocardial homogenates

Poland and Blount[160] reported that myocardial homogenates from running-trained rats had slightly lower oxygen consumption rates during a 2-hour incubation period than did similar homogenates from untrained rats. Lactate production rates during a 40-minute incubation period were also lower in homogenates from trained rats.

Askew, Huston and Dohm[187] found that training increased, but not significantly, the rate at which glycerol-3-phosphate was incorporated into the myocardial glycerolipids; this incorporation occurring principally in the neutral lipids (di- and triglycerides). The myocardial uptake of α-^{14}C-amino isobuturic acid was reportedly decreased by running training[195]. The effects of exercise training on the uptake of ^{3}H-epinephrine by myocardial homogenates has also been studied[232] (previous section).

Isolated myocardial mitochondria

Arcos, Sohal, Sun et al[112] found that, although rats which had undertaken between 140 and 180 hours of swimming training had a 52% increase in mitochondrial mass, when incubated with α-ketoglutarate, succinate or pyruvate as substrates, the oxygen consumption rates of isolated myocardial mitochondria from trained rats were not greater than those of mitochondria from control animals. With glutamate as substrate, there were slight but insignificantly greater oxygen consumption rates by mitochondria from trained animals and the respiratory control ratios in these mitochondria were also reported to be "consistently greater". However myocardial mitochondria isolated from hearts of rats that had trained for between 361 and 490 hours - hearts which did not have increased myocardial mitochondrial masses (section 2.3A) - did not have increased oxygen consumption rates or greater respiratory control ratios when incubated with any of these substrates.

Dohm, Huston and Askew et al[245] found unchanged mitochondrial ADP/O ratios and oxygen uptake (with pyruvate-malate or succinate as substrates) or palmitate oxidation by isolated myocardial mitochondria from running-trained rats. Mitochondria isolated from the skeletal muscles of trained animals had significantly increased values.

These authors also reported that exhaustive exercise decreased the oxygen uptake rates of trained heart mitochondria, but increased these rates in mitochondria from untrained animals. Thus after exhaustive exercise, oxygen uptake rates by myocardial mitochondria from untrained rats were significantly greater than those of trained animals.

Amsterdam, Choquet, Segel et al[224] also found that mitochondria from swimming-trained rats exhibited unchanged mitochondrial respiratory control ratios, respiratory quotients and ADP/O ratios with α-keto-glutarate or glutamate as substrates.

Sordahl, Asimakis, Dowell and Stone[188] reported essentially similar findings because ADP/O ratios, the tightness of respiratory coupling (RCI), and the rates of respiration during active phosphorylation (State 3 QO_2) with either succinate, glutamate/malate or pyruvate/malate as substrates, were unchanged in myocardial mitochondria from running-trained dogs. Studies of myocardial mitochondrial calcium uptake kinetics did however show that mitochondria from trained animals exhibited altered function because they failed to take up calcium from the incubation medium to the same extent as did mitochondria from untrained dogs. Furthermore,mitochondria from only trained animals released some of the exogenous calcium back into the incubation medium. The authors concluded that there may be fewer calcium-binding or transport sites in the mitochondrial membranes of hearts from trained animals.

To investigate this, Asimakis[246] first used ruthenium red, a selective inhibitor of energy-supported calcium transport into mitochondria, and found half-maximum inhibition of calcium uptake occurred at lower concentrations of this agent, in myocardial mitochondria isolated from trained as compared to control dogs. His interpretation was that this indicated that trained hearts had fewer calcium transport sites but those

that were present were used more efficiently. To explain the inability of trained mitochondria to maintain high levels of endogenous calcium, Asimakis suggested that this could be due either to structural alterations in the inner mitochondrial membranes or to reduced levels of intramitochondrial adenine nucleotides. His preliminary studies suggest that mitochondria from running-trained dogs do indeed have reduced levels of intramitochondrial adenine nucleotides, particularly ATP.

Penpargkul, Schwartz and Scheuer[182] also found that swimming-training did not alter mitochondrial ADP/O ratios, RCI or QO_2 (state 4) with glutamate/malate or succinate as substrates. Although QO_2 (state 3) was significantly lower in mitochondria from trained animals, as the mitochondrial protein content of these hearts was increased (section 2.3B), these authors concluded that absolute State 3 oxygen uptake rates were probably not decreased in mitochondria from trained animals. No differences were observed in the rates of either energy-independent calcium binding or respiratory-supported calcium uptake either in the absence of ATP, or at low concentrations of ATP or ADP. In contrast to the 2 studies reviewed above[188,246] these authors did not find that mitochondria from trained animals were unable to retain endogenous calcium. They suggested that this discrepancy might be explained on the basis of differences in either the animal species or the severity, or type of training (swimming versus running) used in the respective studies.

In the final study using this model, Carey, Tipton and Lund[152] isolated mitochondria from hearts of trained and control animals after they had been exposed to between 90 and 150 minutes hypoxia in vivo. No differences between trained and control hearts were found in oxygen uptake rates, RCI, ADP/O ratios or mitochondrial ATPase activities (section 2.3B).

Isolated myocardial sarcoplasmic reticulum.

Penpargkul and his colleagues found that cardiac microsomes enriched in fragmented sarcoplasmic reticulum isolated from swimming-trained male[247] and female[199] rats had significantly greater capacities for both calcium storage and calcium uptake than did microsomes from untrained rats. In female rats, cyclic AMP and protein kinase stimulated calcium uptake to the same degree in both trained and control animals, between which groups there were also no differences in sarcoplasmic reticulum Ca^{++}- and Mg^{++}-activated ATPases (section 2.3B). In contrast, other authors have not found that sarcoplasmic reticulum isolated from hearts of either running-trained dogs[188] or rats[200,248] has increased capacities for calcium uptake, and the most recent studies by Penpargkul and his colleagues[201] seem to be in agreement with these findings. Mg^{++}/Ca^{++}-activated sarcoplasmic reticulum ATPase activities were also unaltered in the running-trained rats[200,201].

Sordahl and his colleagues[188] did however find that the rates of release of the previously bound calcium were significantly less in microsomes from trained hearts and that when the experiments were terminated, microsomes from trained dogs had not yet released all their bound calcium. They concluded that training had altered sarcoplasmic reticulum function, but the way in which these changes influence the regulation of bound calcium remain uncertain.

Isolated perfused rat heart model.

This model which is discussed in detail in Chapter 5, has been used in relatively few studies of myocardial metabolism.

Using potassium-arrested isolated perfused hearts from trained and control animals, Scheuer, Penpargkul and Bhan[249] reported that there were no differences in myocardial uptake rates of glucose or palmitate, or in carbon dioxide production rates during perfusions with either of these substrates. After palmitate perfusions,however, myocardial triglyceride levels were significantly lower and incorporation of the radioactive label into triglyceride significantly higher in trained hearts, suggesting increased myocardial turnover rates in the triglyceride pool in hearts from trained animals.

Moreau, Guilland, Athias et al[250] found increased rates of endogenous triglyceride and exogenous free fatty acid utilization in isolated perfused hearts from swimming-trained rats. Unlike the study reported by Scheuer, Penpargkul and Bhan[249], in this study heart work was not controlled and was significantly greater in hearts from trained animals.

Under conditions of acute global ischaemia, Bersohn and Scheuer[251] found increased rates of lactate release from hearts of swimming-trained rats suggesting that glycolytic rates had been increased by training. During hypoxic perfusions, rates of lactate production, myocardial NADH fluorescence, residual high-energy phosphate stores and myocardial glycogen levels were not different between hearts from trained and control animals[252].

Intact animals.

This model has also been used rather infrequently for metabolic studies.

Injection of isoproterenol into rats in vivo[151] caused a greater decrease in myocardial glycogen levels in hearts, removed 30 minutes later, from swimming-trained than control rats, thereby suggesting increased myocardial sensitivity to the glycogenolytic action of catecholamines.

Studies of noradrenaline turnover by the intact myocardium have been reviewed (section 2.3B). Wollenberger and his colleagues[225,226] found that in response to acute, total myocardial ischaemia, cyclic AMP accumulation was less in hearts from swimming-trained rats than it was in control hearts. Carey, Tipton and Lund[152] reported no differences in ATP, PCr or lactate levels in hearts removed from trained and control animals after periods of hypoxia and ischaemia in vivo. Mitochondrial function under these conditions was also not influenced by prior training (section 2.3C). In contrast, Degenring, Rubio and Berne[155] found lower ATP and higher AMP, adenosine, inosine and hypoxanthine levels in hearts from swimming-trained rats exposed to 5 minutes ischaemia in vivo.

Parizkova and Poledne[253] found that the myocardial uptake of injected radioactively-labelled palmitate in vivo was increased in running-trained rats. In untrained animals, more palmitate was deposited in adipose tissue.

Very few metabolic studies using human subjects have been reported. Using the technique of coronary sinus intubation, Keul[254] studied arterial-venous substrate differences during and after bicycle exercise in 6 trained cyclists. These results were compared to those measured in 27 untrained, age-matched individuals. There were no

differences between trained and control individuals in either oxygen extraction rates, or rates of glucose or free fatty acid uptake either at rest or during exercise. However, rates of lactate extraction were increased in trained cyclists.

Heiss, Barmeyer, Wink et al[255] also found insignificantly greater coronary arterial-venous lactate differences during exercise in trained athletes. No differences in the rates of myocardial glucose or free fatty acid extraction were observed. A major limitation to this study was that the trained group was studied in the fed state, whereas the control group had fasted.

Summary

It is clear from the studies reviewed in this section, that the myocardial metabolic adaptations to exercise training have been poorly investigated. In part this may be explained by the major difficulties posed by any studies measuring heart metabolism in vivo during exercise, and the relative neglect of isolated preparations for these studies.

D. Myocardial functional adaptations to exercise training studied in isolated tissue preparations.

Isolated papillary muscles.

Isolated papillary muscles contracting either isotonically or isometrically have been used by several investigators to study myocardial function after exercise training[256]. In this model, ventricular papillary muscles are excised and mounted by their two ends in a muscle bath; one muscle-end being attached to a force transducer. Electrical depolari-

zation of the cell stimulates the muscle to contract and the following measurements are conventionally recorded:
peak developed tensions (PDT), the maximum rates of tension development (Max +ve dT/dt), the maximum rates of relaxation (Max -ve dT/dt), times from the onset of contraction to peak developed tension (TDT) and the time for tension to fall from its peak to one-half peak developed tension ($\frac{1}{2}$RT). These parameters can be measured either from an initial muscle length that induces the optimum sarcomere length (L max) or, if a tension/length curve is to be studied, at a series of different muscle lengths up to and exceeding L max. Force/velocity curves are generated when the muscle contracts isotonically against different loads. The idealized maximum shortening velocity (V max) is obtained by either graphical or mathematical extrapolation of the force-velocity curve to zero load, whereas the maximum developed force (P_o) is that maximum force developed isometrically when the muscle contracts without shortening. When this model has been used to study the myocardial functional adaptations to exercise training, decreased[256], unchanged[190,224,257,258] and increased[189,193,194,216,244,259] muscle contractility have all been reported.

Thus, Nutter and Fuller[256] found that both papillary muscles and left atrial appendages from hearts of running-trained rats had lower peak developed tensions (PDT) and lower maximum rates of pressure development when contracting isometrically at L max. Contractile dose-response curves to either calcium or norepinephrine were not different between trained and control papillary muscles, nor were their respective functional responses to hypoxia. With detraining, these functional differences were lost.

Grimm, Kubota and Whithorn[190], Williams and Potter[257] and Amsterdam and his colleagues[224,258] have all reported unchanged papillary muscle function from the hearts of respectively, running-trained rats, cats, and swimming-trained rats. In contrast to the findings of Nutter and Fuller[256] the latter authors[224] reported that papillary muscle function was better maintained in trained hearts during hypoxic perfusions.

In contrast to these studies, others have reported enhanced papillary muscle function after training. Whitehorn and Grimmenga[189] reported that isolated columna carnae from swimming-trained rats developed greater tensions at any given diastolic lengths and that their "total work capacity was elevated". Steil, Hansis, Hepp et al[194] found significantly increased peak developed tensions and maximum rates of tension development in trabecular muscle from swimming-trained rats whereas Kammereit, Medugorac, Stell and Jacob[193] also reported significantly greater peak developed tensions, maximum isometric forces and maximum instantaneous powers in trabecular muscles from swimming-trained rats. Tibbits, Koziol, Roberts et al[216] found increased maximum isometric forces in isolated papillary muscles from running-trained rats. In addition, when exposed to 0,5 mM lanthanum, a substance that displaces calcium from the basement membrane and inhibits calcium influx, the time course for developed tension to fall to half P_o was significantly prolonged in the trained hearts. These authors postulated that either
(i) there was more intracellular calcium in the trained hearts, or
(ii) there was more calcium to displace from the extracellular anionic sites or (iii) diffusional barriers reduced the binding of lanthanum to the basement membranes of papillary muscles from trained rats.

Wyatt, Chuck, Rabinowitz et al[244] reported that under control conditions the only difference in contractile function between papillary

muscles from trained and control rats, was that the former developed significantly greater peak forces when contracting isometrically at L max. In response to isoproterenol infusions, the increases in isometric stress and in the rate of rise of stress, and the decreases in time-to-peak force, were all significantly greater in the trained group. Under these experimental conditions adenyl cyclase activity was, as reviewed in the previous section, significantly greater in papillary muscles from trained rats. Others[257] did not find that noradrenaline infusions magnified mechanical differences between trained and control papillary muscles.

Mole[259] reported that peak developed tensions at L max, maximum shortening velocities and maximum isometric tensions were significantly greater in papillary muscles from swimming-trained rats. Isoproterenol increased the maximum rates of tension development in both groups, but the increase was significantly greater in the trained group. Mole contends that the failure of other workers to find significant training-induced changes in papillary muscle function may be because these differences are small and require large numbers of hearts and careful techniques if they are to be statistically-proven.

In none of the studies reviewed above, was there evidence of changes in the passive properties of the isolated papillary muscles.

Isolated retrograde (Langendorff) perfused rat hearts.

In this preparation first described by Langendorff in 1895 (Chapter 4) the aorta of the isolated heart is mounted on a single cannula and the heart is perfused retrogradely via the aorta. Although the heart beats spontaneously the left ventricle does not eject fluid and its

metabolic rate is therefore low.

For a number of reasons, but principally because the working rat heart model (see below) offers far greater scope for the study of myocardial training adaptations, the Langendorff perfusion system has been intensively used only to study the bradycardia of training[229] (section 2.3G). The only study in which this model has been used to study the effects of exercise training on myocardial function is that of Scheuer and Stezoski[252], who investigated the effects of swimming-training on the myocardial metabolic (section 2.3C) and mechanical responses to hypoxia. They found that both during and immediately following hypoxia, rates of coronary flow and of left ventricular pressure development were significantly greater and left ventricular end-diastolic pressures significantly lower, in hearts from trained animals.

Isolated perfused working rat hearts .

In this model (Chapter 4) the heart is initially mounted on an aortic cannula. Thereafter, the left atrium is attached to a second cannula via a pulmonary vein. In this way,the left side of the heart is maintained in continuity with an external perfusion circuit, so that re-circulating fluid which enters the left atrium, passes into the left ventricle from where it is spontaneously ejected into the external circuit. Thus in this model the heart performs external, quantifiable work.

In 1970, Penpargkul and Scheuer[260] showed that isolated working hearts from rats trained with a moderate swimming programme developed greater stroke volumes, calculated stroke work and maximum rates of left ventricular pressure development in response to either rapid atrial pacing or to increases in atrial filling pressures. Rates of myocardial oxygen consumption and coronary flow were also higher in hearts from

trained rats. When the aortic tubing was briefly clamped to induce isovolumic contractions, peak left ventricular pressures and maximum rates of left ventricular pressure development were also significantly higher in hearts from trained animals. These authors concluded that trained hearts had superior pumping capacity and greater aerobic and mechanical reserve due, at least in part, to improved mechanisms of oxygen delivery (i.e increased coronary flow rates).

In the next study from this group, Scheuer and Stezoski[252] found that although stroke volumes and coronary flow rates were not different from control values, peak left ventricular systolic pressures and maximum rates of left ventricular pressure development during both normal and isovolumic beats were, as in their previous study, significantly higher in hearts from trained animals. During hypoxic perfusions, these differences were maintained and the cardiac outputs of trained hearts also became significantly greater.

Subsequently these authors have shown that hearts from swimming-trained rats have enhanced actomyosin and myosin ATPase activities (section 2.3B). To compare the effects of deconditioning on heart function and actomyosin ATPase activities, workers from this group[209] measured both these parameters in control rats, and in rats who had either trained for 8 weeks, or who had undergone 2 weeks' detraining following a similar 8-week training programme. After the initial training period, hearts from trained rats had significantly greater cardiac outputs, rates of coronary flow and myocardial oxygen consumption and maximum rates of left ventricular relaxation (LV_{max}-ve dP/dt). In contrast to their previous studies[252,260], there were no significant inter-group differences in either peak left ventricular systolic pressures or maximum rates of left ventricular pressure development. After 2 weeks' detraining, these

mechanical differences had disappeared with the exception that at the highest atrial filling pressures, rates of coronary flow and myocardial oxygen consumption remained significantly higher in trained hearts. Actomyosin ATPase activities paralleled these differences in heart function, being significantly higher after the 8-week training programme and returning to control values after 2 weeks' detraining. The temporal relationship between these mechanical and biochemical changes was considered suggestive of a causal relationship.

Another possible explanation for the superior function of the trained hearts and one which was not specifically excluded by these studies, was that ventricular dimensions had been altered by training. To specifically study this, Bersohn and Scheuer[261] developed a dye-dilution technique to measure end-diastolic volumes in isolated perfused hearts. When used in conjunction with an electromagnetic flow probe built into the aortic cannula, it was possible to calculate left ventricular volumes, ejection fractions, and the velocities and extents of muscle fibre shortening.

When hearts from trained and control animals were compared using this technique, it was found that at atrial filling pressures up to 15 cm H_2O, end-diastolic volumes were not different between groups. However, mechanical function measured as stroke volumes, ejection fractions, peak left ventricular pressures, peak aortic flows, durations of contractile element shortening and the extents and maximum velocities of circumferential fibre shortening were superior in trained hearts at these atrial filling pressures, indicating enhanced intrinsic myocardial contractility independent of increased left ventricular dimensions (Frank-Starling mechanism). At atrial filling pressures of 20 cm H_2O, end-diastolic volumes were slightly greater in trained hearts and the intergroup differences in mechanical

function were further amplified.

More recently Schaible and Scheuer[262] have used this apparatus to compare myocardial functional adaptations that result from either swimming- or running-training. They found that although the adaptations to either training method were similar, there were certain quantitative and qualitative differences. Thus although the maximum rates of left ventricular relaxation were higher in hearts from swimming-trained than from control rats, there was no difference when values from running-trained and control rats were compared. Thus swimming training may have a specific effect on myocardial relaxation, which is not produced by running.

Despite this difference, stroke work, maximum powers, ejection fractions, maximum velocities and extents of circumferential fibre shortening were significantly increased in hearts from animals trained by either exercise mode. Myocardial efficiencies at atrial filling pressures of 15 and 20 cm H_2O were also significantly greater in hearts from either training group. Despite greater stroke volumes and rates of fibre shortening, maximum rates of left ventricular pressure development were not significantly greater in either trained group than they were in control hearts. The authors considered that this could be explained if the most important mechanical adaptations to training relate to fibre shortening phenomena rather than to changes in rates of pressure development. Thus they suggested that indices of isovolumic contraction might be insensitive measures of training-induced myocardial adaptations.

Bersohn and Scheuer[251] have also compared the effects of global ischaemia on myocardial performance in swimming-trained and control animals. Global myocardial ischaemia was produced by placing in the aortic line, a one-way valve which prevents diastolic coronary flow. During global

ischaemia, hearts from trained animals maintained significantly greater stroke volumes, peak left ventricular systolic pressures and rates of left ventricular relaxation. End-diastolic pressures were also significantly lower in trained hearts. When the obstruction to diastolic coronary flow was removed, rates of coronary flow and myocardial oxygen consumption were significantly greater in hearts from trained animals.

Moreau, Guilland, Athias et al[252] have also reported significantly greater cardiac outputs, left ventricular systolic pressures and coronary flow rates in isolated working hearts from swimming-trained rats.

E. Myocardial functional adaptations to exercise training studied by non-invasive evaluation of the "in situ" heart.

The non-invasive evaluation of the intact heart in trained subjects has largely been limited to humans. The principle study methods have been heart rate measurements by palpation or electrocardiography; auscultation combined with phonocardiography to evaluate cardiac murmurs and additional heart sounds; radiography to assess heart sizes; echocardiography to determine heart sizes and resting and exercising cardiac function; phonocardiography and electrocardiograph to determine systolic time intervals; and respiratory gas analysis during "rebreathing" for the measurement of cardiac outputs (indirect Fick principle).

Clinical examination .

Heart rate .

That highly trained endurance athletes have slow resting heart rates has been realized at least since 1927.[263] In 1929, Bramwell and Ellis[264] reported that resting heart rates of Olympic marathon runners were 15-20 beats/min slower than those of Olympic wrestlers. Similar

observations were made by Hoogerwerf[265] and in 1942, White[266] reported heart rates below 40 beats/min in 3 long distance runners. The lowest documented heart rate in a healthy endurance athlete is 28 beats/min[267]. The mechanisms that are believed to underlie this training bradycardia are discussed in section 2.G.

Cardiac murmurs and added heart sounds.

The frequency with which highly-trained endurance athletes exhibit cardiac murmurs and added cardiac sounds has unfortunately not always been appreciated. The famous American marathoner Clarence de Mar ("Mr. DeMarathon") was prevented from competing in marathon races for 9 years because he had a cardiac murmur[268]. Phonocardiographic evaluation subsequently revealed the presence of an early diastolic 3rd heart sound[101]. At autopsy[101], there were no cardiac abnormalities that would explain this added sound which was considered to be due to ventricular filling. (When he chose to ignore his doctor's advice and returned to marathon running, winning a still unequalled 7 Boston Marathons, De Mar remarked that "I think that the doc must have been listening to his own heart")[268].

A review of the literature reveals that abnormal cardiac sounds are extremely common in endurance athletes. Deutsch and Kauf[263] noted this, and in 1931 Bramwell and Ellis[269] reported a "curious prolongation of the first heart sound, not unlike the crescendo murmur of mitral stenosis" in 12 Olympic athletes, 9 of whom were endurance athletes (3 marathon runners, 3 long distance runners and 3 cyclists). Two marathon runners had a "definite reduplication of the first heart sound at the apex" - a finding not noted in any other athletes. In several other marathoners the first heart sound was reported to be "definitely prolonged".

Gott, Roselle and Crampton[270] reported the presence of a

systolic ejection murmur in a champion oarsman and referred to an American study showing that 40% of young athletes have systolic murmurs. Singh, Crampton and Horgan[271] found that 8 (80%) of 10 high performance athletes referred for the clinical evaluation of possible heart disease had grade 1-2/6 ejection systolic murmurs and third heart sounds, whilst 5 (50%) had fourth heart sounds. Cardiac disease was not present in any of these athletes. Crampton and Lavine[272] reported that 11 (73%) of a 15-member champion lacrosse team had systolic heart murmurs and third heart sounds at rest. One athlete also had an early diastolic mitral murmur. Parker, Londeree, Cupp and Dubiel[273] reported that in a group of 12 long distance runners, all had third heart sounds and 7 (58%) also had fourth heart sounds. In addition, 6 runners (50%) had grade 1-2/6 systolic murmurs in the pulmonary area. Double apex beats were also palpated in 7 runners (58%).

Ikaheimo, Palatsi and Takkunen[274] also studied 12 long distance runners and reported third heart sounds in 8 (75%) and fourth heart sounds in 5 (41%). Of ten sprinters also studied by these authors, 6 (60%) had third and 5 (50%) fourth heart sounds. These authors noted that fourth heart sounds were found only in those athletes who also had electrocardiographic evidence of left atrial hypertrophy.

Using phonocardiography, Roeske, O'Rourke, Klein et al[275] reported that 96% of a group of American professional basketball players had third, and 56% fourth heart sounds. In 12 marathon runners, Zoneraich, Rhee, Zoneraich et al[276] found 2 (16%) with third hearts sounds, and 3 (25%) with apical systolic murmurs confirmed phonocardiographically. Sixteen (66%) of 24 male and female professional ballet dancers[277] had third, and 5 (20%) had fourth heart sounds. Seven (29%) dancers also had grade 1-2/6 early- to mid-systolic murmurs.

Electrocardiography.

Besides resting bradycardia, there is no specific electrocardiographic finding that typifies the heart of the athlete. Rather, the athlete's electrocardiogram has aroused interest because it may frequently contain patterns that closely mimic those seen in ischaemic and other forms of heart disease. These changes are discussed in detail in 2 recent reviews[278,279] and fall beyond the scope of this review.

Although some have suggested that changes in the size of the S wave during exercise may indicate alterations in heart function and therefore potentially distinguish the effects of training on the heart[280], others have found no correlation between electrocardiographic changes during exercise and changes in heart function as measured by radionuclide cineangiography[281].

Radiography.

A number of studies have commented on the radiographic appearance of the hearts of trained sportsmen[263,282-284]. These studies have reported on either diastolic heart sizes (as determined by measurements of the cardiothoracic ratio) or diastolic volumes (measured as the area of the heart shadow taken from postero-anterior and lateral views) in trained persons, usually endurance athletes. With few exceptions these studies have reported increases in both heart size and diastolic volumes. In their manuscript Deutsch and Kauf[263] noted that the principal factors determining heart size in athletes were the type of sport, the age of the athlete, and the number of years in active training. Rowers, swimmers, skiers, runners and soccer players had the largest hearts, but even in these athletes, Deutsch and Kauf considered that the degree of enlargement was not great, nor was it dangerous. They concluded that

this cardiac enlargement was due to ventricular dilatation, because forceful apical thrusts, a sign suggestive of left ventricular hypertrophy, were seldom found in these athletes. Reindell, Roskamm and Steim[285] also concluded that these radiographic changes indicated ventricular dilatation rather than hypertrophy, an interpretation, the correctness of which has recently been confirmed in a number of echocardiographic studies (see following section).

Medved and Medved[284] also noted a relationship between body weight and heart volume in athletes - the heaviest athletes had the largest radiographic heart volumes, whilst Viewig[286] has described a family, 3 of whose members had prominent radiographic left ventricular "hypertrophy" without their ever participating in particularly vigorous endurance exercise. He suggested that there may be a familial predisposition to left ventricular "hypertrophy" occurring in response to even quite mild physical activity.

Echocardiography.

Echocardiographic evaluation of cardiac structural adaptations to exercise training.

The recent introduction of echocardiography has permitted the detailed non-invasive evaluation of the structural cardiac changes that result from exercise training.

Morganrott and his colleagues[283,287] were the first to report echocardiographic studies of the hearts of athletes proficient in either dynamic (running, swimming) or static (shot putting, wrestling) exercises. They found that the hearts of athletes who trained with dynamic exercises had increased left ventricular masses and left ventricular end-diastolic volumes but without increases in left ventricular wall thicknesses. In

contrast, the hearts of athletes trained with static exercises had increased left ventricular masses and increased left ventricular wall thickness but without any changes in left ventricular end-diastolic volumes. These authors concluded that training with dynamic exercises produces cardiac changes similar to those found in pathological conditions associated with chronic volume overloads, whereas the cardiac changes produced by static exercises are compatible with those found in conditions of chronic pressure overload. A number of subsequent reports[273,274,276,288-292] have confirmed these findings in both long distance runners and weight lifters[292].

Echocardiography has also been used to study the hearts of Olympic cross-country skiers[293], childhood swimmers[294], professional basketball players[275], professional cyclists[295,296], male and female professional ballet dancers[277] and female hockey players[297]. All these studies confirm that the principal effect of dynamic exercise training is to increase left ventricular internal dimensions. Left atrial enlargements, larger relative diameters of the aortic roots and greater diameters of the left ventricular outflow tracts have also been reported in long distance runners[274,276,289]. As Ikäheimo, Palatsi and Takkunen[274] found an apparent correlation between left atrial diameter and left ventricular wall mass, they postulated that the left atrial dilatation was caused by reduced left ventricular compliance resulting from left ventricular hypertrophy. Underwood and Schwade have expressed a similar opinion[289].

Longitudinal echocardiographic studies have also been performed to determine how rapidly these training-induced myocardial structural changes appear and alternatively disappear when training is stopped. DeMaria, Neumann, Lee et al[298] showed that a walking/jogging programme performed at 70% of maximum oxygen consumption, 4 hours a week for 11 weeks, caused increased left ventricular end-diastolic dimensions and calculated left ventricular masses. Ehsani, Hagberg and Hickson[299] reported that a

maximum increase in left ventricular end-diastolic dimensions had already resulted after a single week of intensive swimming training and that no further increases occurred during an additional 2 months' training. In contrast, left ventricular posterior wall thicknesses did not increase until the fifth training week but increased no further during the remainder of the training programme. Detraining studies were also performed in a group of 6 distance runners. After 3 weeks without exercise, left ventricular internal dimensions, left ventricular posterior wall thicknesses, and estimated left ventricular masses had all decreased significantly.

In a cross-sectional study which has relevance to these longitudinal studies, Nishimura, Yamada and Kawai[296] compared the echocardiographic and electrocardiographic findings in 3 successive age-groups of professional Japanese cyclists. All 3 groups had enlarged left ventricular end-diastolic dimensions but only the oldest group of cyclists (40 - 49 year olds who had trained intensively since ages 18-20) had significantly increased interventricular septal and left ventricular posterior wall thicknesses. Electrocardiographic abnormalities were also more common in this group as was the incidence of significantly depressed resting left ventricular function. Thus high intensity dynamic exercise maintained through middle-age may cause additional structural adaptations not apparent in short-term longitudinal studies, or in cross-sectional studies of younger athletes.

Echocardiographic evaluation of cardiac function at rest and during exercise.

From the resting echocardiogram, ejection fraction and rates of fibre shortening can be calculated. In trained athletes, ejection

fractions or rates of myocardial fibre shortening have been reported increased[273,288,296,298,300], normal[272-275,289,301,302] or slightly decreased[290,291,296].

Resting stroke volumes were found to be significantly greater in elite distance runners[289], in marathon runners[273,276], in distance runners and swimmers[287], in professional basketball players[275], in cyclists[296], in joggers[298,301-303] and in professional ballet dancers[277] than in non-athletic controls.

In longitudinal studies, Wolfe, Cunningham, Rechnitzer and Nicol[302] found that 3 to 6 months of mild jogging (13 km/week) caused significant increases in resting stroke volumes, due to increased end-diastolic volumes without there being any change in the velocities of circumferential fibre shortening. Fourteen weeks' interval training also increased resting stroke volumes[303].

Recently, dynamic exercise echocardiography has been developed to evaluate heart function during either supine[304] or semi-supine[305] exercise, but this technique has not as yet been used to study athletes. Sugishita and Koseki[304] reported an excellent correlation between cardiac outputs measured echocardiographically and those measured by the conventional dye-dilution technique. Furthermore, changes in mean velocities of left ventricular circumferential fibre shortening during exercise discriminated between both young and older healthy men, and between those with and without mild or severe left ventricular dysfunction.

Weiss, Weisfeldt, Mason et al[305] used this technique to study healthy volunteers during semi-supine exercise and showed that at increasing workloads and heart rates, the mean velocity of myocardial circumferential fibre shortening increased. End-diastolic volumes increased only when heart rates exceeded 110 beats/min, indicating that only at higher heart

rates does the heart utilize the Frank-Starling mechanism to increase stroke volume.

Phonocardiographic/electrocardiographic evaluation of systolic time intervals.

Simultaneous measurement of the electrocardiogram, the carotid pulse and the phonocardiogram allows the calculation of systolic time intervals (STIs) which have been used by a number of investigators as a non-invasive method for evaluating left ventricular function in trained and control individuals[306].

The systolic time intervals most commonly measured are the duration of electromechanical systole ($Q\text{-}S_2$), left ventricular ejection time (LVET), pre-ejection phase (PEP) and diastole (D). The $Q\text{-}S_2$ duration is taken as the time from the onset of ventricular depolarization on the electrocardiogram to the end of contraction as represented by the aortic component of the second heart sound on the phonocardiogram. Left ventricular ejection time is measured from the onset of the carotid upstroke to the nadir of the pulse wave and is taken to represent the period during which blood is ejected from the left ventricle. The duration of the pre-ejection phase (PEP) represents the time from ventricular depolarization to the onset of ejection and is calculated as the difference between total electromechanical systole and left ventricular ejection time. Diastole lasts from the conclusion of ventricular contraction, indicated by the aortic component of the second heart sound, to the onset of the ventricular depolarization in the next complex ($S_2\text{-}Q$).

Most[306] but not all studies[276,289] have shown that at rest, those who participate in regular physical activity have increased durations of electromechanical systole, and longer left ventricular ejection times

and pre-ejection phases. Prolongation of these intervals has been related to the bradycardia of training and is believed to reflect either improved myocardial function or efficiency.

A number of studies have also measured systolic time intervals either during or immediately after exercise in trained and control subjects. All these studies are however open to the criticism that heart rates during exercise were not equal in the two groups, thus the data from each group are not really comparable. To overcome this criticism Wolfe, Cunningham, Davis and Rosenfeld[306] studied both the systolic time intervals and cardiac outputs (measured non-invasively using the CO_2 rebreathing technique described in the following section) at the same heart rates in groups classified as average, moderate and high-fitness. They found that at the same exercising heart rates, the high-fitness group had slightly shorter pre-ejection periods, longer left ventricular ejection times, larger stroke volumes and faster mean systolic ejection rates than did the less-fit subjects. They concluded (i) that the duration of the pre-ejection phase is a poor index of cardiovascular fitness because it differs only slightly in groups showing greatly different cardiovascular fitnesses, (ii) that the longer left ventricular ejection times in the high-fitness group reflected mainly their greater stroke volumes, for which enhanced myocardial performance (calculated as mean ejection rates)* partially compensated as these rates were higher at all heart rates in the high-fitness group, and (iii) that the inter-group differences in systolic time intervals were too small to make them useful indicators of differences in cardiovascular function.

*Footnote: Mean ejection rates were calculated as stroke volumes divided by left ventricular ejection times.

Rebreathing technique for the measurement of cardiac outputs during exercise (indirect Fick principle).

In 1870, Adolph Fick[307] made the observation that blood flow could be calculated according to the equation

$$F = \frac{O}{A - V}$$

where, for example, F equals lung blood flow per unit time, O, the oxygen consumption per unit time, and A and V the oxygen contents of arterial and venous blood respectively. The same formula applies if a foreign substance is introduced into the blood stream either by injection or inhalation, or if a substance is either removed or added to the bloodstream, at a known rate by, for example, the liver or the kidney. In each case, the blood flow to that organ can be calculated from the relation between the rate of its removal or addition, and the difference between the amount of that substance contained in units of arterial and venous blood.

In 1903, Loewy and von Schrötter[308] realized that the lungs could be used as an aerotonometer to measure the oxygen or carbon dioxide tensions of arterial and mixed venous blood so that cardiac output could be measured non-invasively. For both physiological and technical reasons however, most attention has been directed to calculations involving carbon dioxide tensions[309]. Thus arterial partial carbon dioxide pressure (PCO_2) can be calculated from respiratory end-tidal PCO_2, whilst mixed venous PCO_2 is calculated from the exponential rise in the CO_2 content of expired air, during the first few seconds of rebreathing a CO_2:O_2 mixture.

Using the rebreathing technique, Andrew, Guzman and Becklake[310] and Douglas and Becklake[311] reported that after 4 months' hockey training, 12 subjects had reduced heart rates and cardiac outputs at equivalent

submaximum workloads. As oxygen consumption rates were unchanged, arterial-venous oxygen differences during exercise must have increased with training. The latter group also found that during maximum effort, cardiac outputs were unchanged after training. In a group of 10 male cardiac patients, Woodhouse, Hathirat, Jensen et al[312] reported decreased cardiac outputs and increased arterial-venous oxygen differences during submaximum exercise after 8 weeks' training. In cardiac patients enrolled in the Ontario Exercise-Heart Collaborative study Paterson, Shephard, Cunningham et al[313] found that stroke volumes during submaximum exercise increased only after 6 months' high intensity exercise training.

F. Myocardial functional adaptations to exercise training studied by invasive evaluation of the intact heart.

The unanaesthetized chronically-instrumented animal.

Stone and his colleagues[215,239,314] have been the principal investigators to use this model. Their experimental techniques were as follows: prior to the commencement of training, thoracotomies were performed and electromagnetic flow probes positioned around the ascending aortas of dogs. Polyvinyl catheters were passed through the left atrial appendages into the left atria and solid-state pressure transducers were positioned in the left ventricular chambers through incisions in the apices of the dogs' hearts. The catheters and transducer leads were exteriorized and the dogs allowed to recover for 4 weeks before treadmill-training commenced. Heart function was studied in the dogs at rest, and during exercise, when they were untrained, partially- and fully trained.

The principal findings of these studies were as follows:

(i) In the trained state, with the dogs lying quietly on the

laboratory table, peak left ventricular pressures and maximum rates of left ventricular pressure development were significantly reduced, resting heart rates unchanged, and cardiac outputs and stroke volumes significantly increased[314].

(ii) In the trained state, with the dogs standing immediately prior to the treadmill exercise test, heart rates were significantly lower, peak left ventricular pressures unchanged, and cardiac outputs and maximum rates of left ventricular pressure development were significantly increased[314].

(iii) In the trained state during submaximum treadmill exercise, peak left ventricular systolic pressures and maximum rates of left ventricular pressure development, were significantly higher, and heart rates significantly lower. Furthermore, whereas in both the untrained- and the partial-trained states there had been linear relationships between exercising heart rates and maximum rates of left ventricular pressure development, when the fully-trained dogs exercised at heart rates above 180 beats/min, there was a sharp non-linear increase in the maximum rates of left ventricular pressure development[314], a finding which has subsequently been re-confirmed[215]. Cardiac outputs and stroke volumes were also higher at equivalent workloads in the trained state, but of these only one cardiac output value was statistically significant.

(iv) Ventricular function curves were obtained by rapid volume loading with a warm Tyrode's solution infused through an additional catheter positioned in the right atrium by percutaneous puncture of the right external jugular vein. In response to volume loading, ventricular function was depressed in the training

state because, at all mean left atrial pressures above 9 mmHg, heart rates and cardiac outputs rose significantly less in the trained state. Stroke volumes were insignificantly lower in the trained state during volume loading.

(v) Cardiac responses to β-stimulation with four different doses of isoproterenol were also studied in a group of dogs in whom additional flow probes had been placed around their left circumflex coronary arteries[239]. Although there were no differences in the heart rate responses to isoproterenol infusion, cardiac outputs, stroke volumes, circumflex coronary artery flows and maximum rates of left ventricular pressure development were all significantly greater in the trained state.

(vi) In order to measure heart sizes, radio-opaque dye was injected into unanaesthetized dogs, through the indwelling left atrial catheters, under conditions of controlled heart rates. It was found that after 4 weeks' training, ventricular volumes had increased by 30% but there was little change in calculated left ventricular masses. Over the next 4 weeks, left ventricular volumes increased a further 12%, while left ventricular wall thicknesses increased 28%. (A similar pattern of structural changes has also been reported in longitudinal echocardiographic studies[299] - Section 2.3E).

(vii) Rates of coronary flow were also studied at rest, during exercise, and during atrial pacing. It was found that after between 22 and 34 days treadmill-training, atrial pacing caused significantly greater coronary flow rates particularly at the higher heart rates, than had been present in the untrained state. However after a similar training period coronary flow rates

during exercise, were lower at any given workload than they had been in the untrained state. But after a further 4 weeks' training, coronary flow rates during exercise were the same as they had been in the untrained state.

The conclusions from these studies may therefore be re-summarised as:

(a) Cardiac reserve was increased in the trained state - as shown by the greater peak cardiac outputs and stroke volumes after training,

(b) Myocardial contractility, measured as increased maximum rates of left ventricular pressure development and peak left ventricular pressures, in trained dogs during maximum exercise was also increased. Stone[239] postulates that this may be due to a selective increase in the autonomic inotropic influence on the myocardium. The evidence for a selective increase in inotropism was the sudden non-linear increase in maximum rates of left ventricular pressure development at heart rates above 180 beats/min in the trained state. Stone suggests that this could be due to changes in central nervous system command and concludes that the overall effect would be to reduce the energy requirements of the heart during maximum exercise. However as left ventricular end-diastolic volumes were not reported, the alternate possibility that at high heart rates only trained hearts are able to activate the Frank-Starling mechanism (section 2.3F - Radionuclide cineangiography) cannot be excluded.

(c) The reflex response to volume loading was also altered by training because in the trained state, heart rates remained essentially unchanged during volume loading. Although the

exact neural pathways subserving this reflex are unknown, the efferent pathways are believed to run either via the vagi or sympathetic nerves. Stone suggests that increased vagal activity might explain the reduced heart rate response to volume loading in the trained state.

Other studies using the chronically-instrumented dog model have not been as complete as those of Stone and his colleagues, but they are mentioned here for the sake of completeness.

Using this model, Barnard and his colleagues[214,315] have presented preliminary data showing increased maximum cardiac outputs, stroke volumes, and maximum rates of left ventricular pressure development during treadmill exercise in trained dogs. Maximum coronary flow rates were however not increased by training but both at rest, and during submaximum exercise, coronary flows were lower after training.

In a report which does not include an adequate control group, Carew and Covell[316] studied resting heart function in greyhounds who had recently "retired" from racing. During thoracotomies, high fidelity micromanometers were placed in the left ventricle and two ultrasonic crystals were placed on the endocardial surfaces of the minor axis of each left ventricle. Left ventricular function was studied 7 to 50 days later, at rest and in response to volume loading with infused dextran. When these results were compared to values previously obtained in that laboratory in untrained non-greyhound dogs, there were essentially no differences in either maximum rates of left ventricular pressure development or mean rates of circumferential fibre shortening either at rest or during volume loading. In response to volume loading, heart rates increased significantly more in the greyhounds than had previously been observed in other dogs studied in that laboratory. Left ventricular diastolic stiffnesses plotted as the

relationship between volumes and pressures during dextran loading, were not different between the groups.

The open- or closed-chested anaesthetized animal preparation.

Studies of left ventricular function.

A number of workers have used this model to study the effects of exercise training on the heart.

Crews and Aldinger[317] studied left ventricular function using a strain gauge attached to the left ventricle, and showed that developed isometric tensions were not different under control conditions between hearts from swimming-trained and control rats. But in response to increased end-diastolic tension, trained hearts developed greater isometric tensions indicating to the authors that "the physiologically hypertrophied rat heart is capable of a greater work performance than the normal heart". However, in response to adrenaline or isoprenaline infusions, trained hearts developed less isometric tension than did control hearts.

Carey, Tipton and Lund[152] did not find any differences in the resting rates of left ventricular pressure development measured by an interventricular catheter inserted into hearts of swimming-trained and control rats under conditions of either normal oxygenation, or in response to myocardial ischaemia induced by surgical ligation of the anterior descending coronary artery. In response to hypoxia, hearts from trained rats showed a less rapid decline in myocardial performance because they maintained higher heart rates, peak left ventricular systolic pressures and maximum rates of left ventricular pressure development. The heart rate/left ventricular pressure products were also significantly higher in trained hearts at most times during hypoxia. During ischaemia this pattern

was reversed because the mechanical performance of the trained hearts was significantly worse than that of the untrained hearts. Biochemical studies reviewed in section 2.3C were also performed but could not explain the observed functional differences.

Dowell, Cutiletta, Rudnik and Sodt[183] also found no differences in resting myocardial function measured as heart rates, left ventricular systolic pressures, left ventricular end-diastolic pressures, contractility indexes $(dP/dt)P^{-1}$ or cardiac or stroke indexes between hearts from swimming-trained and control female rats. In response to sustained increases in aortic pressures produced by aortic banding for from 1 to 3 days, trained animals maintained or increased myocardial contractility, whereas contractile function was uniformly depressed in hearts from control animals. In response to acute aortic constrictions of short duration (1 - 3 minutes), there were also no inter-group functional differences but when the aortic constrictions were relieved, trained hearts rapidly regained normal cardiac outputs whereas cardiac outputs remained approximately 10% below control values in hearts from control rats.

Cutiletta, Edmiston and Dowell[191] have also studied left ventricular function under conditions of changing pre- and after-loads and during anoxia in running-trained and control female rats. Pre-loads were altered either by infusing or withdrawing blood equivalent to 10% of estimated blood volumes, whereas afterloads were increased by snares placed around the ascending aortas distal to an electromagnetic flow probe. Anoxia was produced by ventilating the animals for one minute with a mixture of 95% N_2:5% CO_2.

In confirmation of their previous study[183], heart function was not different between trained and control animals under conditions of acute increases in afterloads, but when the aortic snares were released, cardiac

indexes returned rapidly to normal in the trained animals but remained depressed in control animals. In response to increased preloads, cardiac indexes and peak flow velocities were insignificantly greater in trained animals, whereas in response to reduced preloads, these 2 parameters were significantly less in control animals. During recovery from anoxia, cardiac indexes increased significantly in the trained animals, due to increased stroke volumes, but were depressed in the control animals. Thus these authors found superior cardiac responses to a variety of interventions in both swimming- and running-trained female rats.

Codini, Yipintsoi and Scheuer[318] likewise found no differences in either peak left ventricular systolic pressures or maximum rates of left ventricular pressure development between hearts from swimming-trained and control animals at rest. When isovolumic contractions were induced by complete aortic constrictions, hearts from trained rats had significantly greater peak left ventricular systolic pressures and maximum rates of left ventricular pressure development. During atrial pacing at heart rates above 400 beats/min, cardiac function was less impaired in hearts from trained rats.

In the most recent study from this group[210], trained and control rats had similar cardiac outputs, stroke volumes, coronary blood flows and myocardial blood flow distributions under control conditions, during hypoxia and during acute volume loading - findings which contrast markedly with their findings in isolated perfused, working rat hearts (section 2.3D).

Sembrowich, Knudson and Gollnick[153] compared resting left ventricular function in hearts from both running-trained and control, normal and myopathic rats. Resting myocardial contractility expressed as contractility index ($dP/dt.P^{-1}$ at dP/dt max), was not different between trained and control normal rats, but in the myopathic group, the trained

rats had less impaired myocardial contractility than did the untrained myopathic rats.

Pfeffer, Ferrell, Pfeffer et al[319] compared the effects of swimming-training on myocardial function at rest and during volume loading with rapid intravenous infusions of Tyrode solution in groups of both normal and spontaneously-hypertensive rats. Under both resting conditions and during volume loading particularly after bilateral vagotomy and with β-receptor antagonism produced by timolol, hearts from trained rats developed significantly greater peak stroke volumes.

Bove, Hultgren, Ritzer and Carey[320] did not find differences in the stroke volume response to acute pressure or volume loading in running-trained and control, close-chested, anaesthetized dogs. They did however observe that left atrial compliance increased after training because left atrial pressures increased less in trained animals in response to equal volume loads, indicating to the authors that "equivalent left atrial pressure changes do not reflect similar states of volume loading in trained and non-trained animals".

Kaplinsky, Hood, McCarthy et al[321] produced acute myocardial infarctions in a group of dogs, some of whom then undertook a running training programme (following section). Cardiac indexes during submaximum exercise were lower in the trained group.

Riedhammer, Rufflenbeul, Weihe and Krayenbühl[322] compared left ventricular function during anaesthesia in running-trained and control dogs using 4 different experimental protocols during which hearts were either unpaced, paced, paced in the presence of cardiac autonomic blockade produced by bilateral vagotomy and propranalol administration, and during acute pressure loading produced by methoxamine infusions. No significant differences were found in any directly measured parameters of left ventri-

cular function, with the exception that during acute pressure loading, end-diastolic pressures increased significantly only in the control dogs. Thus during pressure loading, trained dogs achieved levels of cardiac function equal to those in untrained dogs, without apparently encroaching on the Frank-Starling mechanism, suggesting that the hearts of the trained dogs were functioning at higher levels of contractility.

Cohen, Yipintsoi, Malhotra et al[217] studied resting haemodynamics in trained and control dogs immediately before coronary artery ligation. Although trained dogs had significantly greater resting cardiac outputs, after coronary artery ligation, cardiac outputs were similar in both groups of dogs.

Two additional studies have been performed in which heart function has been assessed either with cinefluorography or cineangiography.

Ritzer, Bove and Lynch[323] used biplane cineangiography to study resting heart function in dogs before and after 10 weeks' running training. They found that after training, resting stroke volumes, peak stroke powers, left ventricular masses and left ventricular end-diastolic volumes all increased but ejection fractions were unchanged. Percentage shortening of the left ventricular walls was significantly increased at rest but not during atrial pacing, whereas velocities of fibre shortening were unchanged both at rest and during pacing.

Wyatt and Mitchell[137] attached radiopaque beads to the endocardia and titanium clips to the epicardia of dogs who subsequently underwent 12 weeks' treadmill running training. After the training period, biplane cinefluorography showed small but significant increases in left ventricular end-diastolic wall thicknesses and left ventricular masses without significant changes in left ventricular end-diastolic volumes. It is not clear why this is the only study that has not reported increased left

ventricular internal dimensions in response to running training (section 2.3E).

One explanation for the failure of most of these studies to show training-induced alterations in heart function - changes which have been clearly documented in the isolated perfused rat heart model, and in the exercising, chronically-instrumented dog preparation - is that in these studies, the hearts have been studied under resting conditions. The possibility that training-induced myocardial adaptations only become apparent when the heart is maximally stressed, provides the rationale for my studies reported in Chapter 5 of this thesis.

Studies of the effects of exercise training on coronary flow and coronary collateral development.

Studies falling under this heading may be subgrouped into those in which the animals were trained after an initial intervention such as coronary artery ligation, and those in which the experimental animals were first trained after which rates of coronary flow were measured either under control conditions or in response to various interventions.
These two different experimental protocols have been chosen to approximate the 2 clinical situations in which it is postulated that exercise training may have a beneficial effect on the coronary circulation - the first providing a model of the effects of physical training in persons with known coronary artery disease, the second, the influence of prior physical training on the coronary blood flow responses to an acute myocardial insult, such as acute myocardial ischaemia.

(1) Studies in which the exercise training commenced after the initial intervention.

Eckstein[324] produced varying degrees of surgical constriction in the circumflex coronary arteries of 96 dogs, who were then divided into control and treadmill-running groups. Two months later, a second thoracotomy was performed and the circumflex coronary arteries were divided immediately distal to the site of prior surgical constriction. Under controlled conditions of aortic pressure, the flow rates through both the constricted and the distal normal arterial ends were measured; the flow rates through the constricted ends were considered to be inversely related to the degree of surgical constriction, whereas flows through the distal normal ends were taken as a measure of the extent of the collateral supply. When both values for each dog were plotted graphically it was found that (i) the greater the degree of surgically-induced circumflex coronary artery narrowing, the greater were the retrograde (collateral) flows and (ii) at any level of surgically-induced arterial constriction, exercised dogs developed greater collateral flows. These authors concluded that moderate and severe arterial narrowing by itself caused increased coronary collateral development proportional to the degree of narrowing, but that exercise training after arterial constriction induced even greater collateralization.

Kaplinsky, Hood, McCarthy[321] produced acute myocardial infarctions in a group of dogs, by surgically occluding their anterior descending coronary arteries. These dogs were then divided into control and treadmill-running groups. After 5 weeks, all dogs undertook an exercise test during which cardiac function was measured (previous section). Immediately thereafter, the dogs were sacrificed and post-mortem coronary angiograms

were performed. There were no differences in either myocardial infarct sizes or in the post-mortem coronary angiographic appearances, suggesting that coronary collateralization had not been increased by short-term training which commenced after a total occlusion had already occurred in a major coronary vessel.

Sanders and his colleagues[325,326] have studied the effects of running training on coronary collateral flows in miniature swine. They found that exercise training did not alter coronary collateral flows either at rest or in response to acute occlusions of the circumflex coronary arteries[325]. However, in response to acute occlusions of the anterior descending coronary arteries which followed a period of exercise training that commenced after a previous more distal (2 cms from its origin) partial left anterior descending occlusion , coronary collateral flow rates increased in both control and trained hearts but were significantly greater in hearts from trained pigs[326]. Thus an acute occlusion which followed an old occlusion by itself increased collateral flow, but when exercise training had been undertaken in the period between the two occlusions, collateral flow increased significantly more in response to the second occlusion.

Heaton, Marr, Capurro et al[327] produced complete occlusions of the anterior descending coronary arteries and partial (60-90%) occlusions of the circumflex coronary arteries in a group of foxhounds, all of whom then underwent studies to determine control values for resting and exercising coronary flow rates. The dogs were then divided into control and treadmill-running groups, and 6 weeks later, coronary flow rates were remeasured during exercise immediately after which the dogs were sacrificed and their hearts excised. It was found that trained dogs had a 39% increase in myocardial blood flows to the collateral flow dependent areas

of the endocardium whereas flows to these areas were unchanged in untrained dogs. Transmural and epicardial blood flows to the collateral-dependent areas were not altered by training. The authors concluded that their results would indicate either training-induced enlargement or proliferation of those collateral channels supplying the endocardium.

Neill and Oxendine[328] produced gradual occlusions of the left circumflex coronary arteries of 33 dogs who were then randomly assigned to either treadmill-running or control groups. Five to 8 weeks later, trained dogs had increased epicardial collateral connections to the occluded left circumflex coronary arteries as judged by higher retrograde blood flows from the distal left circumflex coronary arteries (technique of Eckstein[324]). However, hearts from trained animals were no better able to prevent the development of subendocardial ischaemia in the collateral-dependent areas of the occluded left circumflex arteries during pacing-induced tachycardia. The authors concluded that exercise training may indeed increase coronary collateral development but that such collaterals may be unable to increase coronary flows if, under these conditions, the distal coronary microcirculation is the factor limiting these flows.

It is of interest that angiographically-apparent coronary collaterals in physically-untrained humans with coronary artery disease, do not appear to decrease the manifestations of exercise-induced myocardial ischaemia[329]. Patients with multivessel coronary artery disease and partial collateralization have greater limitations in exercise tolerance than those without such vessels, presumably because in the presence of coronary artery disease, coronary collaterals develop in response to myocardial ischaemia produced by disease progression. The point of Neill and Oxendine's study is that even if coronary collaterals are found to develop after a period of training as reported in the studies of Eckstein[324] and Heaton,

Marr, Capurro et al[327], their functional significance may be open to question.

(2) Studies of the coronary flow responses of trained animals to different interventions.

Response to acute coronary artery occlusions.

Burt and Jackson[330] used the technique of Eckstein and found that exercise training in dogs did not increase coronary collateral flows in response to single acute occlusions of the circumflex coronary arteries and concluded therefore that the normal circumflex coronary arteries carry sufficient reserve to meet the additional flow requirements imposed by exercise. As discussed in the previous section, Sanders, White, Peterson and Bloor[325] drew a similar conclusion from their studies in miniature swine. In a third study to produce similar results, Cohen, Yipintsoi, Malhotra et al[217] reported that after acute occlusions of the left anterior descending coronary arteries of running-trained and control dogs, there were no inter-group differences in either collateral flow rates or in the endocardial to epicardial flow ratios.

Laughlin, Diana and Tipton[331] measured coronary flow rates in the left anterior descending coronary arteries of running-trained and control dog hearts at rest, in response to isoproterenol infusions and during the hyperaemic response to short duration (10 sec) coronary artery occlusions. At rest, rates of coronary flow to the left and right ventricles and to the interventricular septa were significantly greater in trained hearts. In response to short duration coronary artery occlusions, peak hyperaemic flows were also significantly greater in hearts from trained dogs. Furthermore, during isoproterenol infusions, trained hearts maintained significantly greater endocardial to epicardial blood flow ratios in both left and right

ventricles. The authors concluded that trained dogs have increased coronary flow reserves.

Response to hypoxia or pressure or volume loading.

Spear, Koerner and Terjung[332] studied coronary flow rates in running-trained and control rat hearts at rest and during hypoxia. Although resting coronary flow rates were not different between the groups, in response to either hypoxia alone or during hypoxia with mean aortic pressures elevated by methoxamine infusion, coronary flow rates and coronary conductances were significantly greater in hearts from trained animals. These authors suggested that the increased coronary flow rates measured under hypoxic conditions in the trained animals might explain their superior mechanical performance during hypoxia, as reported by Carey, Tipton and Lund[152].

In direct contrast, Yipintsoi, Rosenkrantz, Codini and Scheuer[210] found that coronary flow rates and myocardial blood flow distribution under control conditions and in response to either hypoxia or volume loading, were not different in swimming-trained and control animals studied in the open-chested rat preparation. One possible explanation for the discrepancy between these 2 studies is that different training methods were used (swimming vs running).

In contrast to the study of Laughlin, Diana and Tipton[331], Bove, Hultgren, Ritzer and Carey[320] reported that left ventricular blood flow rates were less in running-trained than in control dogs. In response to volume or pressure loading, total left ventricular flows were the same in both groups. Thus only trained hearts increased myocardial flows in response to volume or pressure loading. Absolute endocardial flow rates were also greater in the trained hearts under these conditions.

Invasive studies in humans.

Coronary sinus intubation.

The myocardial metabolic studies performed by Keul on trained cyclists have been described in section 2.3C.

Heiss, Barmeyer, Winke et al[255] measured coronary flow rates with the argon technique, and reported that at rest and even more so during exercise at equivalent relative workloads (65% of maximum oxygen consumption - $\dot{V}O_2$ max), trained athletes had lower myocardial oxygen consumption rates and lower myocardial blood flows than did untrained controls. The lower coronary flow rates in the trained subjects could not be explained by differences in either diastolic perfusion pressures or in cardiac indices, but were associated with significantly lower heart rate/pressure products. Coronary arterial-venous oxygen differences and myocardial efficiencies were not different between groups.

These authors also compared the coronary blood flow response to dipyridamole, a coronary vasodilator. They found that even at higher doses than those used in the control group, dipyridamole caused a significantly smaller increase in coronary flow rates in the trained group. The authors were unable to explain this finding.

This study shows some of the beneficial haemodynamic adaptation to exercise training. For the same levels of cardiac output either at rest or during exercise, both myocardial oxygen demands and coronary flow rates are reduced in the trained state.

Cardiac output measured invasively.

Submaximum exercise.

As reviewed by Clausen[233,234] invasive studies of cardiac output

during exercise have generally shown that at any given level of oxygen uptake, cardiac outputs measured directly either by the Fick method or by the dye-dilution technique are not altered by training in normal subjects[333-338], whereas they tend to be reduced in persons with clinically-apparent coronary artery disease, who have been physically-trained[60,339-341]. However other studies[342-344] have reported that cardiac outputs during submaximum exercise were reduced after training in persons without heart disease and conversely were unchanged in physically-trained cardiac patients [345,346].

Maximum exercise.

Training-induced increases in maximum cardiac outputs have been reported in virtually all studies of normal persons[335-338,342]. In well-trained athletes, maximum cardiac outputs are greater than they are in sedentary individuals[347] whereas amongst athletes, Ekblom and Hermansen[348] recorded higher cardiac outputs in the good than in the less-good athletes. The highest cardiac output recorded by these investigators was 42,3 litres/min and that for stroke volume was 212 ml. In the famous American marathoner Clarence De Mar (section 2.3A and 2.3E), Bock,Vancaulert, Dill et al[349] recorded a peak stroke volume of 217 ml. For comparison,peak stroke volumes measured in Drs. David Dill and A.V. Bock were 150 and 128 ml respectively. Reference to other studies in which cardiac outputs have been measured during exercise in trained athletes and sedentary individuals may be found in the paper by Hanson and Tabakin[350].

Adaptations in persons with coronary artery disease.

That the cardiovascular adaptations to exercise training may differ between those with and those without heart disease,specifically

coronary artery disease, is suggested by those studies showing reduced cardiac outputs at given submaximum oxygen uptakes in cardiac patients after training (see previous section). However it is now clear that in persons with coronary artery disease who have suffered acute myocardial infarction, overall cardiovascular function continually alters in response to changes in the disease state, and that such changes may mask less-marked adaptations that result from training. Thus Rousseau, Dergé, Messin et al[346] reported that in the first 6 months after acute myocardial infarction, stroke volumes during submaximum exercise increased in untrained control patients to the same extent as they did in subjects undergoing a training programme. This indicates that an early increase in submaximum cardiac output is part of the spontaneous evolution of cardiac function after acute myocardial infarction.

Bruce, Kusumi and Frederick[351] have extended these observations. They compared two groups of post-myocardial infarction patients who had participated in a training programme; the one group included those subjects whose age-related fall in maximum oxygen consumption was less than predicted, the second group comprising those subjects who showed a more rapid decline in $\dot{V}O_2$ max than would have been expected purely due to aging. During progressive treadmill exercise after varying periods of training, the former group showed increases in arterial oxygen content and arterial-venous oxygen differences and small reductions in heart rates, cardiac outputs and pulmonary artery pressures. However in the latter group, during submaximum exercise after "training", there were much larger (11%) decreases in cardiac outputs, due entirely to reduced stroke volumes and much greater (14%) increases in arterial-venous oxygen differences. This study therefore indicates that when reduced stroke volumes and increased arterial-venous oxygen differences are measured in "trained" cardiac patients during

exercise, they may not indicate a training effect, but may reflect a further adaptation (increased peripheral oxygen extraction) to disease progression causing reduced exercising stroke volumes.

Peripheral factors and training-induced alterations in cardiac output during exercise.

The question may be asked whether a training-induced increase in maximum cardiac output indicates that the heart itself has increased its functional capacity. Clausen and his colleagues[233,234] were the first to develop an experimental protocol that directly evaluates this question.

In their studies, two different groups of subjects were trained to exercise on bicycle-ergometers using either their legs or their arms. After the training programme had been completed, their cardiovascular responses measured during exercise involving either the "trained" or the "untrained" limbs were compared to those measured prior to training. It was reasoned that if training induces adaptations only in the heart, then any training-induced changes in the cardiovascular response to exercise must be independent of which limbs are tested after the training programme. In contrast, if the altered cardiovascular responses to exercise after training were dependent on peripheral (non-cardiac) adaptations, then exercise with either the "trained" or "untrained" limbs would elicit different responses. If this were true, after training,the cardiovascular responses to exercise with the limbs that had not participated in the training programme, would be the same as that recorded for those limbs in the initial pre-training test. During exercise with the "untrained" limbs therefore, the heart would react as if it too, was untrained. The results of these studies show that training-induced increases in maximum cardiac outputs cannot be accepted as prima facie evidence for enhanced

myocardial performance, if the re-testing was performed on the limbs that were actively trained.

The authors found that when measured during exercise after training, maximum cardiac outputs and maximum whole body oxygen consumption rates were increased regardless of whether the "trained" or "untrained" limbs were tested, but these increases resulted from fundamentally-different mechanisms.

During exercise with "trained" legs, the increased cardiac outputs occurred in association with either unchanged or reduced total peripheral resistances. Therefore it was not possible to state that the increased cardiac outputs measured during exercise with the "trained" limbs indicates improved myocardial function because, after training, the heart may be pumping against a reduced pressure load. However, when the "untrained" arms were re-tested during exercise after the legs had been trained, it was found that maximum cardiac output increased, despite increased peripheral resistance. This, according to Clausen, may be clearest evidence so far available for a training-induced improvement in human myocardial function.

There is however, additional evidence suggesting enhanced myocardial function even when the trained limbs are tested. This follows from the observation that when, during a training programme, each leg is exercised separately on a bicycle ergometer (i.e. although both legs perform the same amount of exercise and are therefore equally trained, they are always exercised in sequence and never simultaneously), the absolute training-induced increase in $\dot{V}O_2$ max is less if both legs are tested together, than if each leg is tested separately. The explanation offered by Clausen, is that even maximum exercise with a single leg does not exceed the inherent pumping capacity of the "untrained" heart. Therefore during training with

a single leg there is insufficient cardiac stress to improve myocardial function. The result is that when both legs are exercised simultaneously, the "untrained" heart is unable to fully utilize, in each leg at the same time, the locally-created potential for increased muscle blood flow and oxygen uptake.

Clausen concludes that for there to be absolute increases in maximum cardiac outputs during exercise with the trained muscles, myocardial function must be sufficiently increased to take advantage of the peripherally-located potential for increased muscle blood flows. Failure of adequate myocardial adaptation will cause a centrally-mediated sympathetic vasoconstrictor restraint to override this peripheral vasodilator potential, thereby ensuring that during maximum exercise, the pumping capacity of the heart is not exceeded, so that arterial blood pressure and central haemodynamics are maintained at the expense of increased blood flows to the active muscles.

Left ventricular function, coronary artery anatomy and coronary blood flows measured by ventriculography, coronary angiography and coronary sinus intubation in physically-trained persons with coronary artery disease.

Studies using coronary angiography and left ventriculography have failed to show that exercise training increases myocardial function either at rest[61,62,352-353] or during exercise[62] or increases coronary collateral development in persons with coronary artery disease[60-67].

In addition to these studies, several investigators have used both direct and indirect methods in an attempt to determine whether exercise training can reduce the extent of exercise-induced myocardial ischaemia in persons with coronary artery disease. By comparing the double or triple

products (heart rate x systolic arterial blood pressure x ejection time - used as indirect measures of myocardial oxygen consumption rates) with the extent of exertional ST-segment depression and the subjective feeling of ischaemic chest pain (the angina threshold) before and after exercise training, various authors have tried to determine whether exercise training reduces either the degree of ST segment depression or prevents the development of angina at given levels of myocardial oxygen consumption. Detry and Bruce[354], Nolewajka, Kostuk, Rechnitzer and Cunningham[61] and Costill, Branam, Moore et al[355] all found that the relationship between the double product and the degree of ST-segment depression was not altered by training indicating that the extent of myocardial ischaemia had not been reduced by training. In fact, after training, the patients developed greater ST-segment depressions at the point at which they first reported chest pain, indicating that exercise training had increased their angina thresholds. Likewise, Sim and Neil[356] and Redwood, Rosing and Epstein[357] have reported increased double or triple products at the angina thresholds after training, but their electrocardiographic data were inadequate to evaluate whether these were associated with either increased or decreased ST-segment changes. More recently, Ehsani, Heath, Hagberg and Holloszy[358] and Raffo, Luksic, Kappagoda et al[359] have presented data showing increased rate/pressure products at 0,1 mV ST-segment depression after training.

Two studies have directly measured coronary flows after training in persons with coronary artery disease. Sim and Neil[356] studied coronary flows during pacing-induced angina, before and after 11-15 weeks' training. They found that pacing-induced angina thresholds were not altered by training nor, at subanginal pacing levels, were the coronary flow rates, directly measured myocardial oxygen consumption rates, or coronary arterial-venous oxygen differences. Furthermore, patients in whom pacing had caused myo-

cardial lactate release before training, also showed this abnormality after training.

The authors concluded that exercise conditioning had probably not altered myocardial oxygenation, at least during pacing-induced angina. These studies were unfortunately not repeated during exercise. Therefore, there are 3 possible reasons why, in their study, exercise training increased the triple product at the angina threshold - either there was increased myocardial oxygenation only during exercise but not during atrial pacing,or after training there was an altered relationship between the externally-measured and true myocardial oxygen consumption rates, or the perception of angina was reduced so that after training only more severe myocardial ischaemia was perceived as angina.

Recently Ferguson, Côté, Gauthier and Bourassa[360] have performed precise quantitative studies of coronary sinus blood flows and myocardial oxygen consumption rates at rest and during submaximum exercise in cardiac patients before and after training. At rest after training, there were significant reductions only in heart rates, but during submaximum exercise, as would be expected, there were reductions in heart rates and in systolic, diastolic and mean arterial blood pressures. Systolic ejection times were significantly increased. Because of the reduced rate/pressure products and therefore the lower myocardial oxygen consumption rates, coronary sinus blood flows were reduced and coronary vascular resistances increased after training. But at either the same heart rates or at the maximum symptom-limited work loads, coronary sinus blood flows, myocardial oxygen consumptions, rate/pressure products and coronary arterial-venous oxygen differences were not significantly altered by training.

These authors therefore conclude that the training-induced increase in exercise tolerance of patients with angina pectoris does not

necessarily depend on augmented myocardial oxygenation, but is related to reduced coronary flow requirements for any absolute external work load. They stressed however that the thermodilution technique used by them does not measure regional blood flows. Thus,the findings of reduced total coronary blood flows during submaximum exercise after training does not necessarily indicate reduced flows to the ischaemic zone.

Radionuclide cineangiography[361,362].

The first studies using this technique to evaluate the athlete's heart or the effects of exercise training on heart function have recently been reported.

Rerych, Scholz, Sabiston and Jones[363] reported that maximum heart rates and ejection fractions were unaltered by 6 months' swimming training, but maximum cardiac outputs increased due to increased end-diastolic volumes. The highest cardiac output measured in this study was 56,6 litres/min in an Olympic silver medallist, a value considerably greater than those recorded by Ekblom and Hermansen using dye-dilution techniques[348].

Although resting heart rates and ejection fractions were reduced, cardiac outputs were maintained by increased end-diastolic volumes. Thus training improved overall heart function, in part through greater activation of the Frank-Starling mechanism during exercise.

In direct contrast, Bar-Shlomo, Morch, Feiglin et al[364] found that both trained athletes and untrained individuals increased ejection fractions to the same extent during exercise, but each group achieved this by different mechanisms. Whereas the untrained group increased end-diastolic volumes during exercise, thereby utilizing the Frank-Starling

mechanism to increase ejection fractions, the athletes' hearts increased their exercising ejection fractions through decreased end-systolic volumes.

Preliminary studies in cardiac patients[365,366] have shown that exercise training may be associated with impairment, normalization or improvement of ejection fractions measured during exercise. Exercise-induced wall motion abnormalities remained unchanged after training[366].

G. Studies to elucidate the mechanisms underlying the bradycardia of training.

That physically-trained persons exhibit resting bradycardia, is a fundamental observation (section 2.3E), yet an adequate explanation for this adaptation remains elusive[103]. A number of possible mechanisms have been proposed and these are:

(i) that the bradycardia is genetically-determined,

(ii) that it results from a change in the intrinsic sino-atrial discharge frequency,

(iii) that it is due to an alteration in the balance between the sympathetic and parasympathetic neural outflows to the heart,

(iv) that it results from changes in autonomic receptor sensitivities or their intramyocardial messengers, or

(v) that it is due to changes in the action potential.

The scientific evidence for each viewpoint will be discussed separately.

The genetic explanation for the bradycardia of training.

Although there may be some genetic component to the bradycardia found in superior athletes, there are now sufficient studies confirming that bradycardia is a biological adaptation resulting from chronic exercise

training[103]. Thus reductions in resting and submaximum exercising heart rates are 2 of the most well-defined indicators of a training effect.

For reasons that are not clear, sleeping heart rates are not reduced by training[367,368]. It is also of interest that neonatal heart cells isolated from the offspring of exercised pregnant rats exhibit slower beating rates[369] than heart cells cultured from mothers who were inactive during pregnancy.

Changes in the intrinsic sino-atrial discharge frequencies.

The intrinsic discharge frequency of the sino-atrial node can be measured either in isolated preparations, such as isolated right atria or isolated perfused rat hearts, or in intact mammals by autonomic nervous system blockade produced by the simultaneous administration of adequate doses of atropine and propranolol.

Isolated right atrial preparations.

Hughson, Sutton, Fitzgerald and Jones[220] and Smit and El-Hage[370] have reported reduced intrinsic sino-atrial frequencies of isolated atria from running-trained rats. Furthermore, exercised rats which also received atropine medication during training, had even lower intrinsic sino-atrial frequencies than did rats which exercised without such medication. As atropine blocks the effects of the parasympathetic nervous system on the heart, this finding indicates that the training-induced reduction in intrinsic sino-atrial frequencies is not dependent on increased parasympathetic activity resulting from training. Furthermore, carbachol, a stimulator of parasympathetic activity, not only failed by itself to reduce intrinsic sino-atrial activities in atria from control rats, but

it also prevented the expected training-induced reduction in intrinsic sino-atrial frequencies.

In common with others (section 2.3B) these authors also found increased myocardial potassium concentrations in hearts from trained rats. They hypothesized that if sino-atrial node potassium concentrations were also increased, this would cause wider transmembrane potassium gradients in the trained hearts, since serum potassium levels in these animals were not different from controls. Increased transmembrane potassium gradients would theoretically cause greater negative resting potentials which in turn would cause slower intrinsic sino-atrial frequencies. This would however not explain reduced intrinsic sino-atrial frequencies in other groups of their rats in which myocardial potassium levels were not increased.

Isolated perfused rat heart model.

In vitro intrinsic heart rates measured in isolated perfused hearts from trained rats which had significant resting bradycardias *in vivo*, were not different from those of control rats[229]. The reasons why isolated rat atria from trained animals should show reduced heart rates, whereas isolated perfused hearts also from trained animals do not, is not known. This may possibly be related to residual catecholamine activity known to be present in isolated perfused hearts[371].

Intact animals with blockade of the autonomic nervous system.

In both man[372,373] and rats[374,375], intrinsic heart rates measured *in vivo* by double pharmacological blockade of the parasympathetic and sympathetic nervous systems are reduced by training. Highly-trained athletes also have lower heart rates during autonomic blockade than do untrained subjects[376]. In contrast, Lewis, Thompson, Areskog et al[368]

did not find that training reduced intrinsic heart rates.

Sigvardsson, Svanfeldt and Kilbom[377] measured intrinsic heart rates in trained and control rats which were pithed, sympathectomized and vagotomized immediately prior to the heart rate measurements. One group of trained rats was also chemically-sympathectomized during the training programme. It was found that sympathectomy prevented the fall in intrinsic heart rates that occurred in trained normal rats. Thus, during the training period, an intact adrenergic but not a cholinergic system[220] is necessary for there to be a fall in the intrinsic heart rate.

Alterations in the balance between the sympathetic and parasympathetic neural outflows to the heart.

Studies to determine changes in the balance between the sympathetic and parasympathetic nervous influences on the heart can obviously only be performed in intact animals.

Parasympathetic system.

Such studies have shown that after submaximum doses of atropine, trained animals[241,242,378,379] develop less cardiac acceleration at rest than do control animals. When maximum doses of atropine are used, no significant differences in resting heart rates between trained and control rats[380] and dogs[381] were found. Scheuer and Tipton[103] interpret these results to indicate that training has either increased myocardial acetylcholine levels (section 2.3B) or reduced myocardial sensitivity to submaximum doses of atropine. In response to injections with cholinesterase-inhibitors such as neostigmine, trained rats had significantly lower resting heart rates than did untrained rats[241,242], a finding which is compatible with increased resting parasympathetic tone in the trained state.

During submaximum exercise in rats[382] and humans[383], atropine injections increased heart rates less after training, indicating that increased parasympathetic tone alone cannot explain the training-induced reductions in exercising heart rates.

Sympathetic system.

Many studies indicate that training alters resting sympathetic tone. Dowell and Tipton[384] found that propranolol caused greater reductions in resting heart rates in control than in trained rats, suggesting higher resting sympathetic tones in the untrained animals. Similarly Lin and Horvath[374] calculated that resting sympathetic tone was 15% greater in untrained rats.

In addition, during exercise, β-receptor blockade with propranolol has less effect on submaximum exercising heart rates after training in both rats[382] and humans[383]. As increased sympathetic drive is the major factor increasing exercising heart rates[385], this finding suggests either that sympathetic tone during exercise is reduced or that parasympathetic tone is increased (see above), or that adrenergic receptor sensitivities have been reduced. Reduced serum[235] and coronary sinus catecholamine[236] levels after training would be compatible with the former possibility.

On the other hand, in the presence of complete autonomic blockade, heart rates in humans[368,373,383] and rats[382] are reduced at all levels of exercise after training. Indeed,in a group of athletes, autonomic blockade had essentially no effect on exercising heart rates[376]. Furthermore,after chemical sympathectomy produced by 6-hydroxy-dopamine, the lower exercising heart rates of the trained rats persisted[382]. Thus Lewis, Thompson, Areskog et al[368] and Ekblom, Kilbom, Malmfors et al[382] concluded that changes in neither the sympathetic nor parasympathetic

system, nor the adrenergic nerves, nor the circulating catecholamines can adequately explain the reduced exercising heart rates after training.

Changes in autonomic receptor sensitivities or their intra-myocardial messengers.

Isolated adrenergic receptors.

Williams[386] found no differences between swimming-trained and control rats in either myocardial β-adrenergic or muscarinic cholinergic receptor numbers or affinities, and concluded that altered release of neurotransmitters (section 2.3B) in the heart, rather than altered responsiveness of the sinus node cells, was the more likely explanation for the bradycardia of training.

Isolated right atrial tissue.

Hughson, Sutton, Fitzgerald and Jones[220] reported that the beating rate responses to both acetylcholine and norepinephrine infusions were the same in atria isolated from trained and control rats. However, because atria from trained rats had lower intrinsic beating frequencies, the maximum beating responses to norepinephrine were reduced in the atria from trained rats. Furthermore, there was a correlation between peak beating rates achieved during norepinephrine infusions, and the intrinsic sino-atrial frequencies. Thus ,atria with lower intrinsic sino-atrial frequencies had lower maximum responses to norepinephrine stimulation. The authors concluded that those intra-cellular electrophysiological factors which determine the intrinsic sino-atrial frequencies also limit their maximum beating frequencies.

Smith and El-Hage[370] found that the chronotropic responses

to atropine were greatly increased in atria from trained rats, and suggested that training-induced increases in atrial acetylcholine levels would explain this finding.

Isolated perfused rat heart model.

Using this model, Tipton, Matthes, Tcheng et al[229] found that isolated hearts from trained animals exhibited significantly less cardiac acceleration with submaximum doses of atropine whereas,when perfused with either neostigmine (an inhibitor of acetylcholinesterase and cholinesterase), acetylcholine or propranolol, the reduction in heart rates was the same in hearts from either group. Isoproterenol infusion caused significantly greater heart rate increases in hearts isolated from trained animals.

From these findings,the authors concluded that the bradycardia of training is the result of increased acetylcholine and decreased norepinephrine concentrations at the cardiac receptor sites. Training had also apparently increased myocardial β-receptor sensitivities.

Intact animals.

Dowell and Tipton[384] and Harri and Narvola[387] have reported that isoproterenol infusions caused significantly greater increases in heart rates in trained animals. In response to noradrenaline infusions, blood pressures rose more in trained animals[387]. Interestingly, in this latter study, chronic β-receptor blockade,like chemical sympathectomy[377], prevented the development of both the resting bradycardia and the cardiac hypertrophy produced by training.

Pavlik, Hegyi and Frenkl[388] performed similar studies in trained rats. In agreement with the 2 previous studies they found that

the chronotropic response to isoproterenol was significantly greater in hearts from trained animals but that β-receptor blockade abolished this difference. Furthermore, in response to alpha-receptor stimulation, trained animals showed significantly smaller blood pressure rises whereas the α-antagonist, phenoxybenzamine, caused significantly greater blood pressures falls in trained animals. Also, during α-receptor blockade, the magnitude of the blood pressure response to noradrenaline was reduced in trained animals so that,at higher drug concentrations, the intergroup difference in the blood pressure response were lost. Thus, α-receptor blockade abolished the enhanced pressor response to α-stimulation and β-receptor blockade, the enhanced chronotropic response to β-stimulation in trained rats.

These authors therefore concluded that those reactions brought about by α-adrenergic stimulation were reduced, whereas those caused by β-stimulation were increased in trained animals. Similarly, sensitivity to α-receptor antagonism was increased and that to β-receptor antagonism reduced in trained animals. For 3 reasons, they did not believe that these differences could be explained on the basis of altered receptor sensitivities because:

(1) The alpha- and beta-receptor responsivenesses altered in different directions.

(2) The direction of altered responsiveness to either stimulation or inhibition were in opposite directions. Had the receptors altered their sensitivities, one would have expected reduced reactivity to alpha-stimulation to have been associated with reduced sensitivity to alpha-blockade.

(3) There were no differences in the amount of antagonist required to produce complete receptor blockade in either group.

These authors postulated that after training, the setting of the autonomic nervous system is altered in the direction of reduced sympathetic activity. Thus, β-receptor blockade after training cannot further reduce the resting heart rate, but the possible range for the heart rate response to isoproterenol is increased. An opposite response affected the α-receptors; at rest α-receptor activities were increased after training so that although trained rats were less responsive to α-stimulation, they exhibited increased responsiveness to α-blockade. In conflict with this interpretation are the findings of Harri and Narvola[387] and Le Blanc, Boulay, Dulac et al[389], that norepinephrine infusions caused greater absolute blood pressure increased in trained rats and humans respectively.

The complexity of these studies is shown by the in vivo findings that the hormonal changes to histamine and various stress-invoking procedures are different in control and trained animals[390]. Thus when studies were performed in "pithed" animals, the heart rate and blood pressure responses to noradrenaline were the same in trained and control rats[377].

Changes in the action potential.

Although the action potential has not been measured in the in situ heart, the monophasic action potential provides some relevant information. Brorson, Conradson, Olsson and Varnauskas[391] measured right atrial effective refractory periods and monophasic action potentials in 8 volunteers before and after 6 weeks' training. They found that the duration of the monophasic action potential at 50% and 90% of repolarization increased in 5 subjects both during spontaneous sinus rhythm and during pacing at the same rates studied before training. The

relative repolarization rate during phase 5 decreased in 5 of 7 subjects.

The authors suggested that an increased rate of depolarization in phase 0 of the atrial action potential and a longer action potential duration might be explained on the basis of increased intracellular potassium levels.

Tibbits, Roberts and Barnard[392] measured the resting membrane potentials and the action potential durations in isolated left ventricular fibres of trained animals. When perfused with Hank's solution containing a normal calcium concentration, there were no intergroup differences in either of these parameters. However, at high perfusate calcium concentrations (3 mM), the plateau phase of the action potential was significantly prolonged in the trained rats. Since the plateau phase is partially a result of the inward calcium current, they postulated that this finding was compatible with enhanced calcium influx to the myofilaments, as suggested by their previous findings[216].

SUMMARY AND CLINICAL RELEVANCE.

It is clear that, at present, there is no single explanation for the bradycardia of training. Rather, there is evidence to suggest that exercise training reduces the sino-atrial discharge frequency, it alters the balance between the sympathetic and parasympathetic neural outflows to the heart, and it alters the concentrations of intra-myocardial neurotransmitters.

The clinical relevance of this condition is that extreme training bradycardia can induce a sick sinus-like syndrome with hypotensive episodes[393]. By reducing the athlete's training load and prescribing atropine, these symptoms can be effectively eliminated.

H. OVERALL SUMMARY AND CONCLUSIONS.

The studies reviewed in section 2.3 of this chapter show that there is little absolute consensus about the effects of exercise training on the heart.

It is clear that, in laboratory animals, absolute heart weights may or may not increase in response to training, and that even in highly-trained humans, heart weights are not greatly increased. However, there is firm echocardiographic evidence that the hearts of endurance-trained athletes show increased internal dimensions and that there may also be increased aortic root and left ventricular outflow tract diameters. The histological changes that accompany these adaptations are poorly understood, in particular, because of conflicting results from animal studies.

From studies in both the isolated perfused working rat heart model and in the chronically-instrumented dog preparation, there is good evidence that, when maximally-stressed, the trained animal heart has superior mechanical performance. When studied at rest, however, these changes are usually not apparent.

Due largely to the extreme technical difficulties posed by performing similar studies in humans, and therefore the resulting lack of convincing data, it can not yet be stated with absolute conviction that the heart of the physically-trained human also has superior mechanical performance. Thus, although maximum cardiac outputs are increased by exercise training and reach very high values in superior human athletes, there are also peripheral changes, in particular a reduced peripheral vascular resistance, which may be the more important factor explaining increased cardiac outputs of trained humans. Another possibility is that venous return to the heart limits cardiac output and that the greater cardiac outputs in trained humans reflects their ability to maintain higher rates

of venous return during maximum exercise. Whether radionuclide cine-angiography or other novel non-invasive techniques will provide final answers to these questions remains to be seen.

The myocardial metabolic and biochemical adaptations to exercise training are, if anything, even less well understood than are the mechanical adaptations. There is a general consensus that exercise training does not increase the myocardial capacity for aerobic metabolism, although it may increase myocardial glycolytic capacity. Increased activities of the myocardial contractile protein ATP hydrolyzing enzymes, and increased rates of calcium uptake by isolated sarcoplasmic reticulum have been reported in hearts of animals subjected to swimming but not to running training. There is also a considerable body of evidence suggesting that myocardial metabolism of catecholamines and acetylcholine is altered by training. Only two studies have compared the myocardial metabolism of trained and untrained humans and neither were able to show training-induced adaptations.

Although animal studies have suggested that training may increase the extent of the coronary vascular bed in both normal hearts and in those with experimental coronary artery occlusions, no human studies have yet shown that exercise training increases either coronary collateral development or coronary flow rates either in the absence or in the presence of coronary atherosclerosis. Whether or not exercise training can reduce the rate of progression of established coronary atherosclerosis remains to be established.

Finally, the precise explanation for the resting bradycardia of training remains to be established.

CHAPTER 3.

EXERCISE AND CORONARY ATHEROSCLEROSIS.

STUDIES OF HEART DISEASE IN MARATHON RUNNERS.

Unforeseeing one! Yes he fought on the Marathon day:
so, when Persia was dust, all cried "To Akropolis!
Run Pheidippides, one race more! The meed is thy due!"
"Athens is saved, thank Pan", go shout!
He flung down his shield, and Athens was stubble again,
a field which a fire runs through,
Till in he broke "Rejoice, we conquer!". Like wine through clay,
Joy in his blood bursting his heart, he died - the bliss!

"Pheidippides"

Robert Browning, 1879.

3.1 INTRODUCTION.

The epidemiological and experimental evidence that exercise might play a role in preventing coronary atherosclerosis or its lethal sequelae was reviewed in Chapter 2 (section 2.2). It was shown that there is some evidence for either postulate. Thus, exercise may prevent or retard the development of coronary atherosclerosis. Alternatively, it may act to protect the myocardium against the development of lethal arrhythmias. The "Bassler hypothesis" provided an initial test of these different possibilities.

The "Bassler hypothesis" first formulated in 1972 (section 2.2C), proposes that marathon running may be an effective way of preventing death from coronary atherosclerosis because "a search of the literature has failed to document a single death due to coronary atherosclerosis amongst marathoners of any age[71]". So stated, the hypothesis does not differentiate between a protective effect of exercise training either on coronary atherosclerosis or on the myocardium.

However, a major weakness of this hypothesis was that its validity rested on a negative supposition, namely on the absence of evidence that could disprove it. Because the hypothesis argued that, as coronary atherosclerosis had never been documented in marathon runners, then that group of sportsmen must be immune to the disease. But an equally plausible explanation for this apparent lack of coronary atherosclerosis in marathon runners could be that no previous research had studied this specific question in sufficient depth. I therefore set out to determine whether heart disease could be found in South African marathon (42 km) and ultra-marathon (> 42 km) runners.

Accordingly, beginning in 1975, circulars were sent to South

African marathon runners informing them of my interest in this subject. This established a groundswell of interest and resulted in a number of referrals and consultations with runners who have cardiac disease. In addition, any cases of sudden exercise-related deaths in marathon runners reported in the media (Figure 3.1) were immediately investigated. In this way, full clinical details on each case were collected and, where possible, the hearts of deceased runners were subjected to meticulous autopsy examinations by Professor Alan Rose (Department of Pathology, University of Cape Town).

At the time of writing, 4 cases of sudden death and 5 of acute myocardial infarction in marathon runners have been thoroughly researched. In 5 other cases of sudden death in marathon runners, no autopsies were performed and the data are therefore incomplete. Much of this material has already been published[96,394-398], but is published here in greater detail for posterity.

A TRIBUTE TO DENIS REED

Runner dies during race

Man, 37 dies in marathon

DURBAN. — A 37-year-old man, Mr Denis Hersey, died yesterday after collapsing during the 42 km Toti marathon.

Mr Hersey of Sanctuary Place, Moseley, collapsed after completing about 10 km of the run, which began at Hutchinson Park in Amanzimtoti early yesterday.

He was taken by ambulance to the Kynock Hospital at Umbogintwini, where he was certified dead on arrival.

Road runner dies in race

Weekend Argus Correspondent

JOHANNESBURG. — A 44-year-old runner from Orange Grove, Mr Jim Park, collapsed and died during the 25 km Simba Road Race in Kempton Park today.

Mr Park, an experienced runner with Jeppe Athletic Club who had completed the Comrades Marathon, collapsed after completing 20 km of the course.

He was given medical attention at the watering points every 5 km along the route, but died in the ambulance on the way to the Kempton Park Hospital.

● Edgar Cloete, winner of the Sacred Marathon in Athens, with 15 year old Don Bainick, of Rustenburg.

● Father and son in the Sacred Marathon: James Coker, 45, and John, 12.

Heart attack didn't stop Daan

,Perdfris' man in marathon dood

"This Wasn't Supposed to Happen"

by Allen Schauffler

Figure 3·1

Newspaper reports of sudden deaths and heart attacks in marathon runners.
These reports provided the initial information necessary for the identification and investigation of some of the cases reported in this chapter.

3.1 ACUTE MYOCARDIAL INFARCTION IN MARATHON RUNNERS.

A. Cases with clinical diagnoses but without coronary angiographic evidence.

CASE 1. (Case 5 in Reference 96).

A white male,37 years old in 1976, who was a nonsmoker and who had no family history of heart disease, began jogging regularly in 1967 in order to improve his health and to lose weight. A routine medical examination performed in 1972 for insurance purposes revealed T-wave inversions in the inferior electrocardiographic leads (2, 3 & AVF). The patient was told that he had the billowing mitral leaflet syndrome (Barlow's Syndrome) and he received insurance only at a loaded premium.

Despite this, the patient became a serious long-distance runner and in 1973 and 1974 completed the 90 km Comrades Marathon (Fig. 3.2) in 7 hr 17 min and 7 hr 20 min respectively. His best standard (42 km) marathon time during that period was 2 hr 51 min. For both those years he ran approximately 3200 km in training. He trained even harder for the 1975 Comrades Marathon and,during that race, he passed the 45 km halfway mark in the good time of 3 hr 19 min. However,after he had run a further 10 km, he suddenly felt very tired, nauseous and noted that he began to sweat profusely. After walking for 20 minutes he was again able to run. This sequence recurred 3 times, but the patient did not have chest pain nor did he vomit.

He completed the race in 7 hr 33 min but,as he continued to feel ill after the race, he was referred for admission to a local hospital. Overnight he was treated with intravenous fluids and the following morning was found to be grossly oedematous with a jugular venous pressure raised

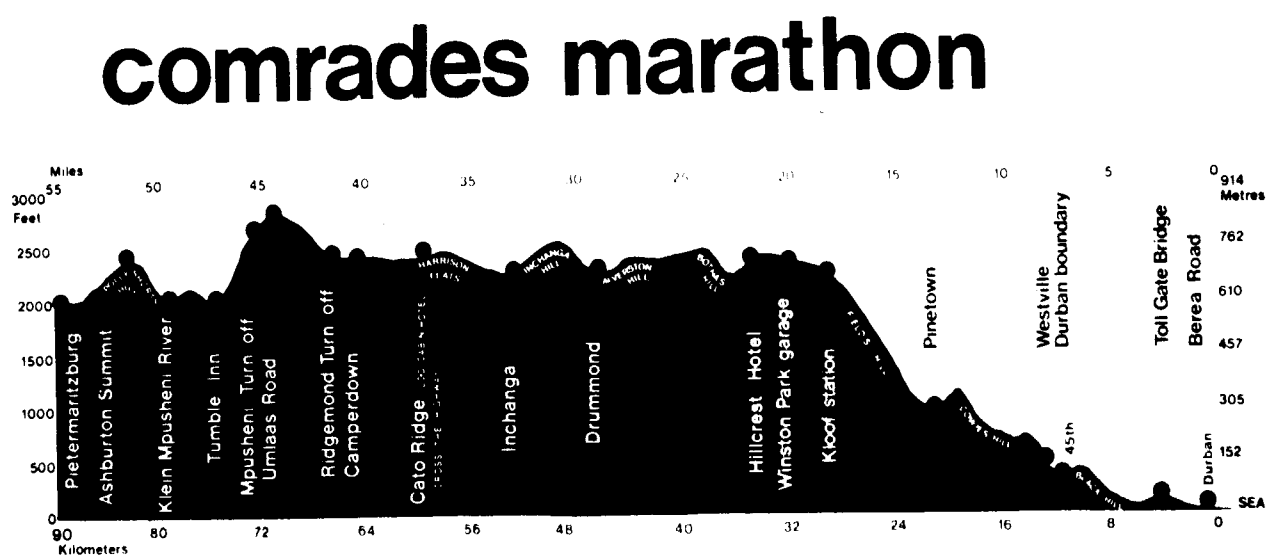

Figure 3.2

Profile of the course on which the 90 km Comrades Marathon is run.

The race, run in opposite directions in alternate years, was established in 1921 so that future "members of the Comrades Association should for one day suffer in memory for their Comrades (of the Great War) who did not return."

The Comrades Marathon Story.
Morris Alexander, 1976.

8 cm above the manubrium sterni, a third heart sound, dullness to percussion at both lung bases, and a 3-cm hepatomegaly with gross abdominal ascites. An electrocardiogram performed at that time showed an acute inferior myocardial infarction and serial enzyme changes were compatible with that diagnosis. The patient responded well to conservative therapy and was discharged from hospital 11 days later.

He was seen at Groote Schuur Hospital in December 1976, 19 months after his initial hospitalization,by which time he was jogging up to 32 km a week. He no longer competed in marathon races. The clinical examination was entirely normal, there being no stigmata of hyperlipidemia, the patient's blood pressure was 130/90 mmHg , the heart sounds were normal and all pulses were present. The chest Xray was normal, and the electrocardiogram showed an old transmural inferior myocardial infarction (Fig.3.3). Echocardiography revealed all the heart chambers to be of normal size; the movement of the mitral valve was also considered to be normal and not compatible with a diagnosis of Barlow's Syndrome. During an exercise stress test, segmental ST depression first appeared at a heart rate of 160 beats/min and became progressively more severe in the V5 lead up to a heart rate of 190 beats/min. Immediately after exercise, ST segment depression was present in leads V4, V5 and V6. The patient declined to have coronary angiography performed.

The possibility that this patient had mitral valve prolapse rather than coronary artery disease has been raised[399]. In the absence of coronary angiographic evidence, the issue cannot be finally resolved. It should,however,be noted that the echocardiogram was normal, whereas the exercise stress test revealed electrocardiographic ST segment depression in the lateral leads, but in the absence of chest pain.

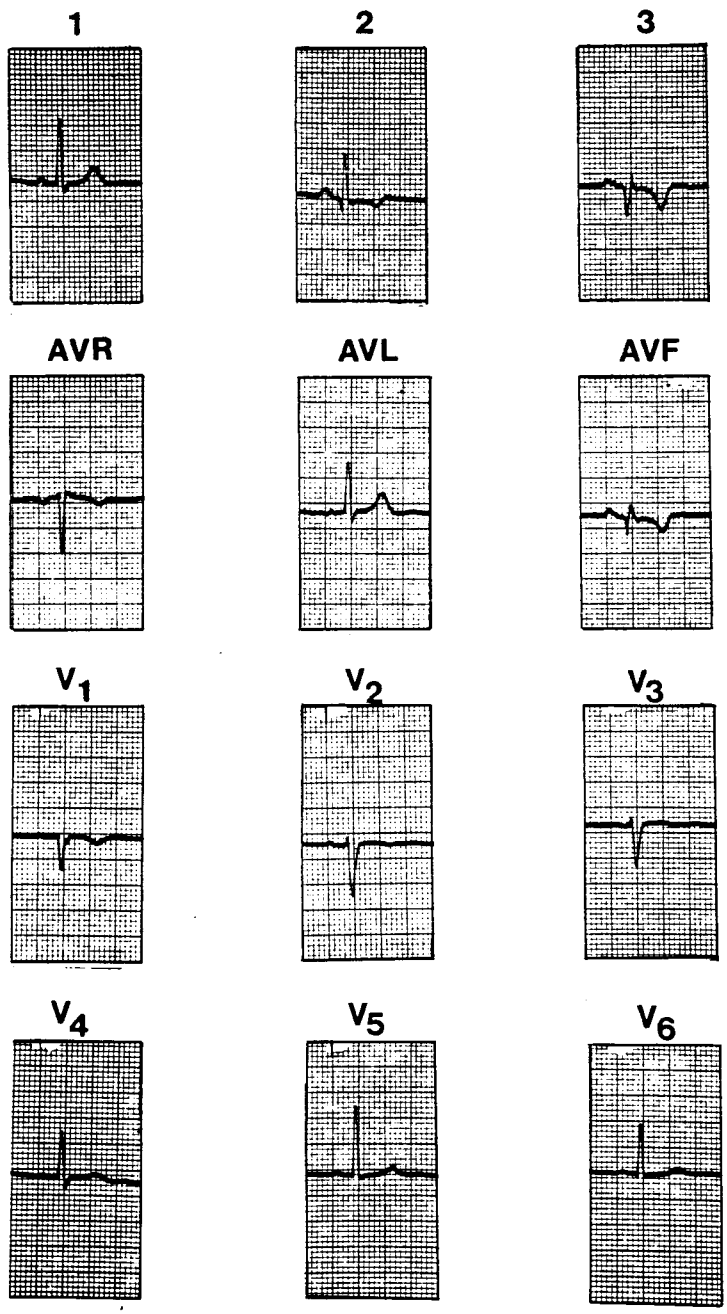

Figure 3.3

Case 1. Resting electrocardiogram showing Q waves in leads 2, 3 and AVF.

CASE 2.

A 41 year old white male was hospitalized with an acute transmural inferior myocardial infarction in November 1978. He had been running long distance races for 2 years, during which time he had run 7 standard (42 km) marathons (best time 3 hours 46 min) and in May 1978, the 90 km Comrades Marathon in 10 hours 29 min. During that period he had trained on most days, covering distances between 8 and 20 km and had run as much as 150 km/week before the Comrades Marathon.

There was a strong family history of heart disease: the patient's father had died at age 57 after suffering a number of heart attacks, the first at age 40 years. In addition, two paternal uncles had also died from acute myocardial infarctions. The patient had smoked about 25 cigarettes a day for 20 years but had stopped in 1975,shortly before taking up running. There were no other significant features in the medical history.

On 18th November 1978, whilst on a normal training run, the patient experienced severe nausea which forced him to stop running and to walk home. On the following day,whilst at work, the patient experienced typical ischaemic chest pain and was admitted to a local hospital. The electrocardiogram showed a normal sinus rhythm with a heart rate of 70 beats/minute and there were pathological Q waves with ST segment elevation in leads 2, 3 and AVF. Serum enzymes were elevated on that and the following day with SGOT 115 U/L (normal values up to 42), LDH 865 U/L (normal values up to 560) and CPK 217 U/L (normal values up to 100). Fasting blood cholesterol was normal (222 mg/100ml - normal up to 250),whereas triglycerides were elevated (168 mg/100 ml - normal up to 150). The blood sugar was normal (82 mg/100 ml),but the serum uric acid level was elevated

at 7,3 mg/100 ml (normal up to 6,5).

The hospital course was uneventful, and the patient was discharged on propranalol 40 mg 8-hourly. Eight weeks later, submaximum exercise stress test up to a heart rate of 136 beats/min showed a normal blood pressure response to exercise, and there were neither electrocardiographic ST segment changes nor arrhythmias. The resting 9 lead electrocardiogram recorded prior to that test shows transmural inferior myocardial infarction (Fig. 3.4). The patient was admitted to a cardiac exercise rehabilitation programme and progressed to the point where he was again able to run 42 km in training.

In March 1980, however, he suffered a further myocardial infarction from which he has recovered well and continues to jog.

B. Cases with clinical diagnoses and with coronary angiographic evidence.

CASE 3. (Case 3 in reference 96).

A white male, 48 years old in 1976, had been physically active throughout his life and was a nonsmoker. There was a family history of heart disease, his father had died suddenly at the age of 60 and a paternal uncle had died of "heart failure".

There was no past history of significance. He had been a long distance runner for 10 years and during this period had completed seven 90 km Comrades Marathons and more than 32 000 km in training. After completing the 1975 Comrades Marathon in 9¾ hours, he maintained a heavy training schedule until the end of October 1975 when he travelled to Greece to run the "Sacred Marathon" - on the course over which the legendary marathon (see poem "Pheidippides" by Robert Browning) was run. For 6 weeks

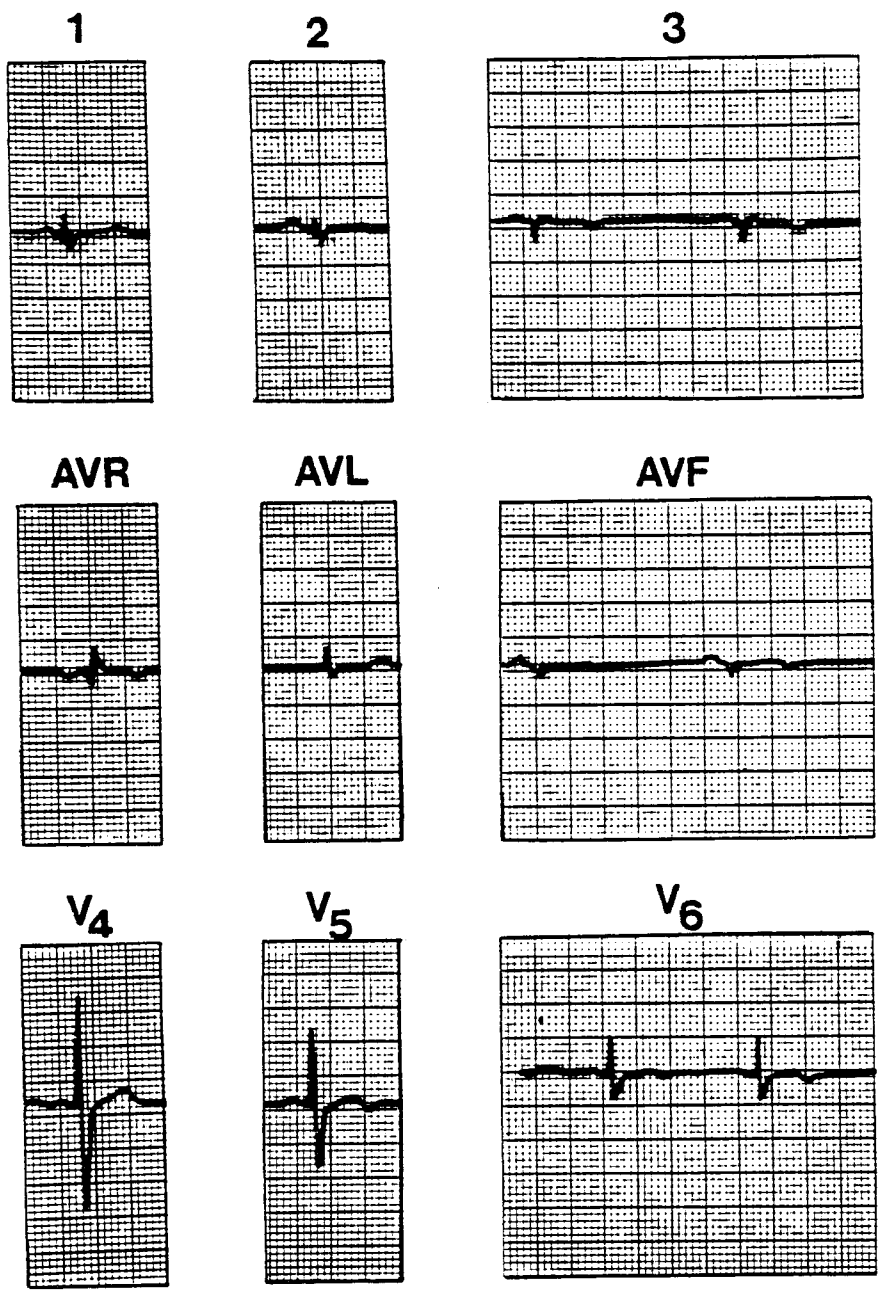

Figure 3.4

Case 2. Resting 9 lead electrocardiogram showing Q waves in leads 2,3 and AVF. Each tracing in this electrocardiogram has been enhanced.

prior to this event he had been training 128-144 km a week, including 42 km runs at weekends. He had never previously trained this intensively.

The Sacred Marathon was run in adverse environmental conditions, it being dry and hot, with the race only starting at 1 p.m. Facilities to supply the runners with fluid replacement were inadequate and,in contrast to his usual practice, the patient was forced to drink very little fluid during the first 32 km of the race. At about this point he began to feel nauseous and was aware of a dull pain in the epigastrium. The pain forced him to walk and he soon began to vomit. There was no chest pain and,despite the discomfort and regular episodes of vomiting, the patient insisted on completing the last 10 km of the course, which he did in 2 hours.

After the race the patient returned to his hotel where he continued to vomit and was unable to eat or drink. Four hours after finishing the marathon, the patient was pain free and was seen by a marathon-running physician who prescribed an antiemetic to control the vomiting and advised the patient to drink fluids as he was dehydrated. During the night the patient passed urine but remained unwell. For the next 24 hours his condition remained unchanged, but on the evening of the following day the epigastric pain radiated up both sides of the chest and into the back. A cardiologist was called and a diagnosis of acute myocardial infarction was made. The patient was admitted to hospital where the electrocardiogram showed acute anteroseptal myocardial infarction. The patient was given 2 litres of intravenous fluid rapidly as he was dehydrated and anuric. This treatment relieved both the epigastric and the chest pains. His further course was uncomplicated and 4 days later he flew back to South Africa where on arrival he was admitted to hospital for a further 8 days.

Four days later he began walking 6-8 km a day, and 3 weeks later he recommenced jogging. In January 1976 he was running 42 km a week and by the beginning of March he had doubled this.

In August 1976, he was admitted to Groote Schuur Hospital for cardiac evaluation. Systemic interrogation did not reveal any additional features. On physical examination there were no stigmata of hyperlipidemia. The patient's somatotype was endomesomorphic (4:5½:2 - Heath Carter) and he had 22,5% body fat estimated by skinfold measurements. The resting pulse rate was 52 beats/minute and the blood pressure 130/90 mmHg. While the apex beat was not displaced, there was a palpable fourth heart sound and,on auscultation at the apex in the lateral decubitus position, a systolic click and a late systolic murmur were audible. Phonocardiography at the mitral area showed third and fourth heart sounds and an intermittent nonejection click. No definite systolic murmur was recorded.

The electrocardiogram showed the pattern of an old transmural anterior myocardial infarction with lateral T wave abnormality (Fig. 3.5). The chest X-ray showed mild cardiomegaly with a cardiothoracic ratio of 52%. The lung fields were clear. A glucose tolerance test was within normal limits. The fasting lipogram showed normal values for cholesterol (268 mg/100 ml) and triglycerides (107 mg/100ml). Echocardiography showed that the left ventricular internal diameter was increased, the percentage of fractional shortening was normal and there was no increased thickening of the posterior left ventricular wall.

At cardiac catheterization, the resting left ventricular end-diastolic pressure was markedly elevated (30 mmHg). Coronary angiography showed a 2 cm area of 50% - 75% narrowing in the mid-portion of the left anterior descending artery, but with good filling of the normal distal vessel (Fig. 3.6), the mainstem and circumflex vessels were free of disease

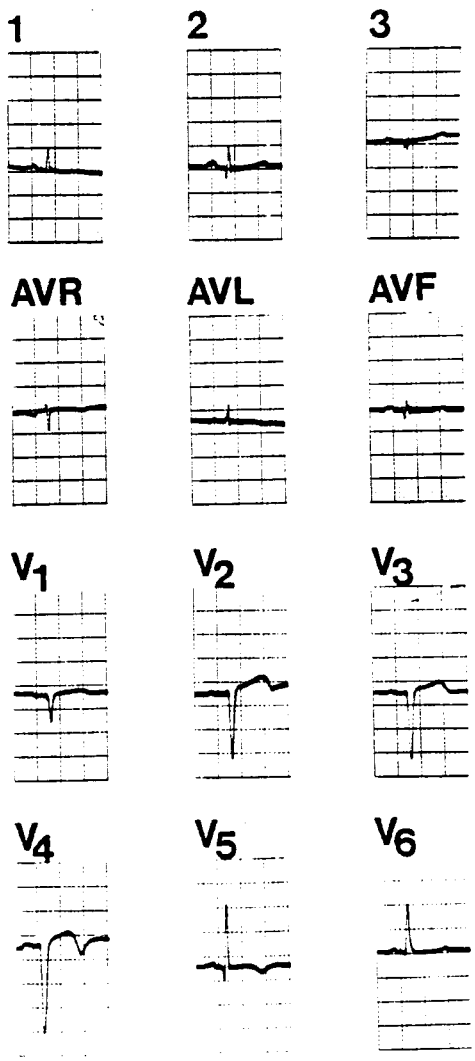

Figure 3.5

Case 3. Resting electrocardiogram showing Q waves and loss of R amplitude in V leads, and lateral T wave abnormality.

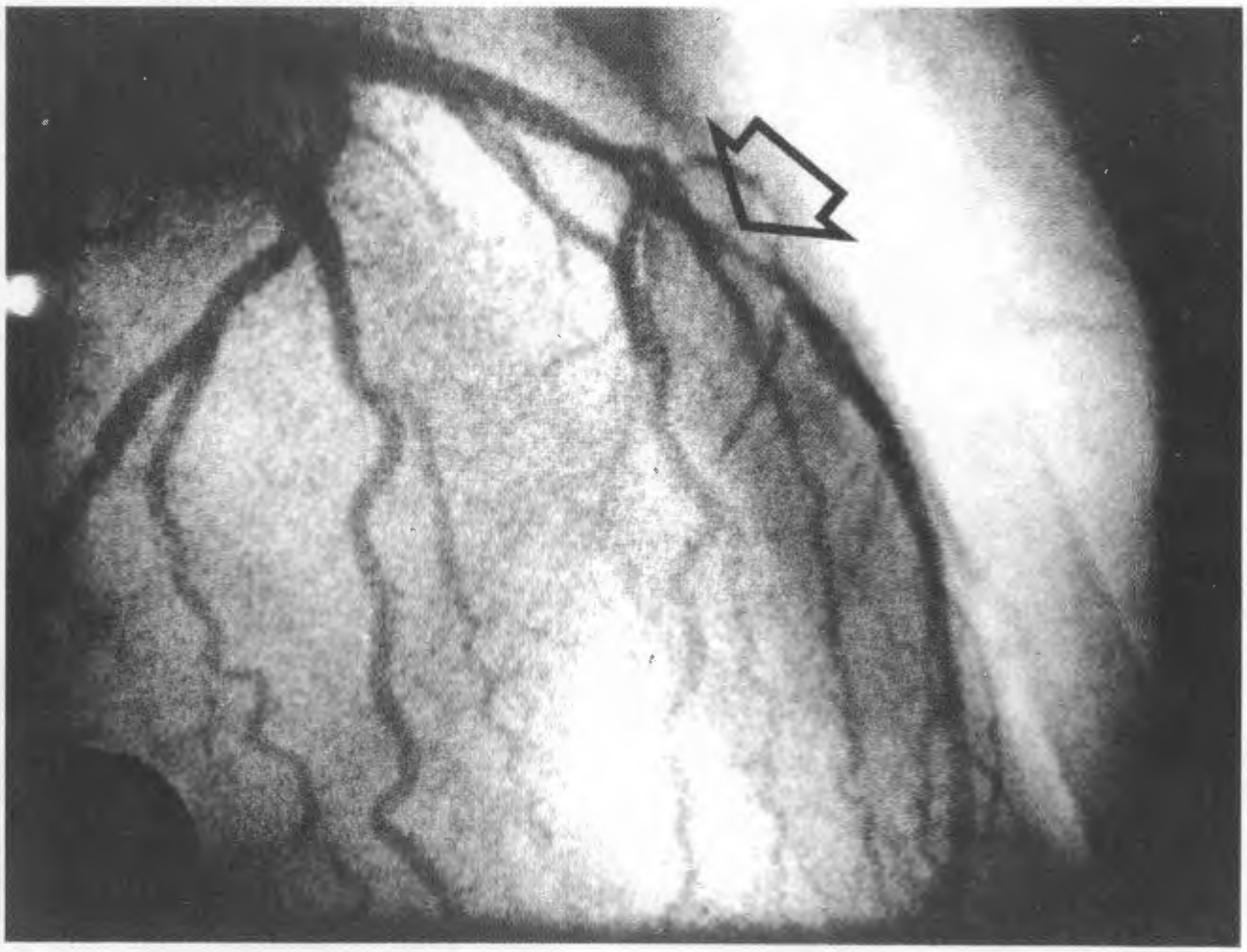

Figure 3.6

Case 3. Left coronary angiogram in right anterior oblique projection, showing a 2 cm segment (arrowed) of the anterior descending coronary artery with 50 - 75% narrowing and good distal filling.

and the right coronary artery was unobstructed, dominant, and of large calibre. The left ventricular angiogram showed an enlarged left ventricle with an extensive akinetic area involving the apical, anterolateral and inferior surfaces.

In the 4 years since these investigations, the patient has returned to full training and has again run the Comrades Marathon. He is presently asymptomatic.

CASE 4. (Case 6 in reference 96)

A 46 year old white male athlete was first seen in 1972 with a complaint of pain developing in the left groin during running. He was fully examined, including an electrocardiogram (Fig. 3.7) and no cardiovascular abnormality was reported.

The patient was next seen in December 1974 with the complaint that,while running 5 days previously, he had developed severe anterior chest pain. Despite the chest pain, he had refused to stop running and had run on 3 of the following 4 days, including a 16 km time trial. On each occasion he had experienced chest pain. An electrocardiogram at that time showed changes of anteroseptal myocardial infarction (Fig. 3.7). The patient refused hospitalization. He was allowed to rest at home but persisted in physical activity, including one period in which he mowed his lawn for 5 hours with severe chest pain.

In February 1975, an exercise electrocardiogram, a glucose tolerance test, a fasting lipogram, and serum uric acid estimation were all normal. The patient was allowed to start careful jogging but was advised against any future competitive running. However,in September 1975, he completed a 27 km road race without difficulty and in April 1976 he ran 150 km during a 3-day race.

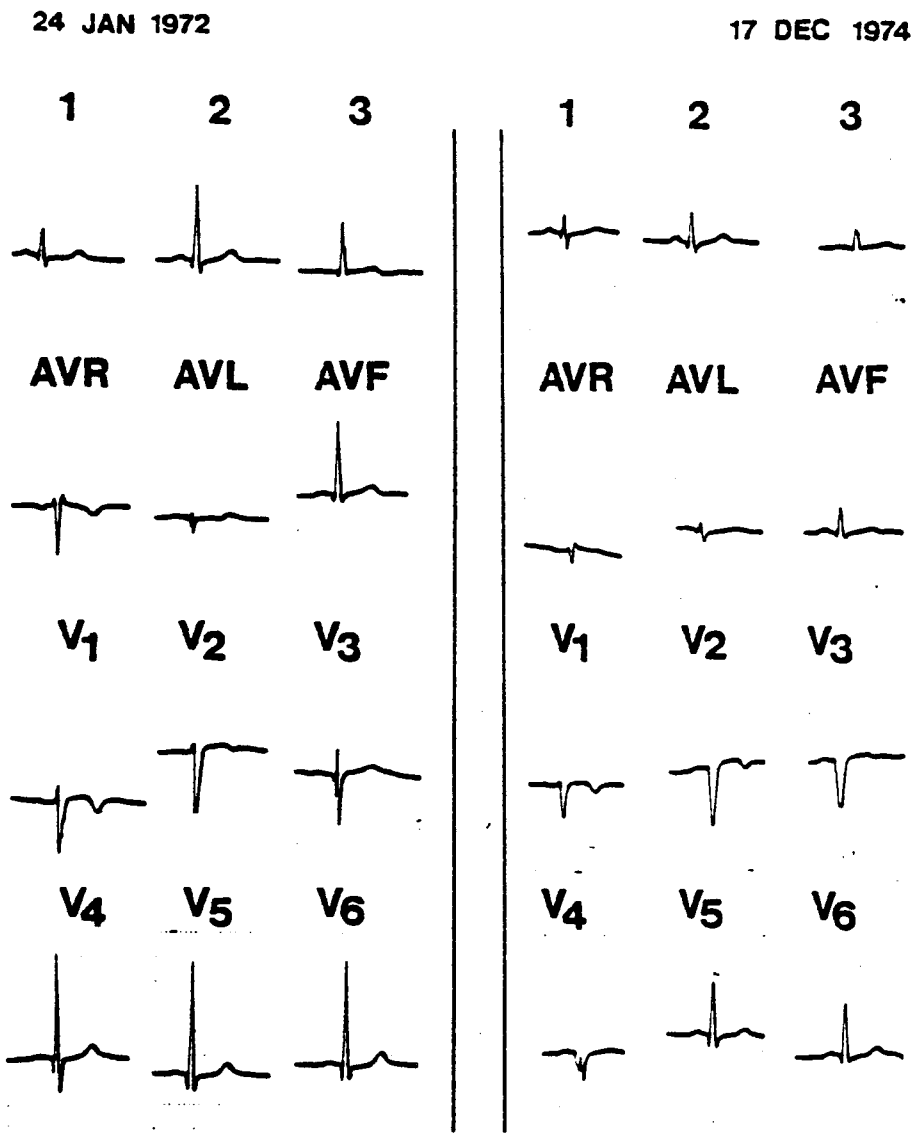

Figure 3.7

Case 4. Resting electrocardiograms taken 2 years apart. The 1972 tracing shows diminished R waves in V_1 and V_2 and narrow Q waves in V_3 suggestive of old anterior infarction. The more recent tracing, taken 4 days after the onset of chest pain, shows Q waves with loss of R waves in $V_2 - V_4$.

In December 1976, 2 years after the original diagnosis of acute myocardial infarction, the patient was admitted to Groote Schuur Hospital for cardiac evaluation as he wished to run another Comrades Marathon.

Special interrogation revealed that the patient was known to have retinitis pigmentosa, leaving him with moderately impaired vision. There was a family history of heart disease, his father dying suddenly from a heart attack at the age of 51. His mother had died at the age of 53 from diabetes and was a carrier of an X-linked gene for retinitis pigmentosa. The patient was a teetotaler and had never smoked. He had always been extremely physically active, representing his Province at swimming whilst still a schoolboy. Failing vision forced him to turn to rowing, and in 1958 he was selected to represent South Africa as an oarsman at the Empire Games. In 1960 he took up long distance running and between 1961 and 1974 had completed 14 consecutive Comrades Marathons and had run more than 42 000 km in training.

Systemic interrogation revealed only that the patient frequently developed exertional chest pain, particularly when running uphill or before he had "warmed up". When on the flat he was able to "run through" his chest pain, but was often reduced to a walk on the uphill. On physical examination, there were no stigmata of hyperlipidemia. The pulse rate was 65 beats/min with the occasional irregular beat, and the blood pressure was 130/90 mmHg. The cardiac apex was impalpable and there were no abnormal auscultatory findings. All peripheral pulses,except the dorsalis pedis on both sides,were present and there were no arterial bruits.

The chest X-ray was normal and the resting ECG was unchanged from that shown in Fig. 3.7. An effort electrocardiogram was unchanged up to a heart rate of 174 beats/min.

Left heart catheterization showed all the pressures to be normal.

Coronary angiography revealed a dominant right coronary artery with mild proximal disease and a 70% area of narrowing in the posterior descending artery (Fig. 3.8). The distal left anterior descending coronary artery was seen to fill retrogradely.

The mainstem left coronary artery was free of disease but the circumflex artery had mild, diffuse disease (Fig. 3.9). The left anterior descending artery was narrowed by approximately 80% in at least three areas of its proximal and midportions with the distal vessel filling late (Fig. 3.9). A large diagonal branch, supplying a significant area of myocardium, was present (Fig. 3.9). The left ventricular angiogram showed a large akinetic area involving the antero-lateral, apical and diaphragmatic segments with good contraction of the inferobasal and antero-basal segments (Fig. 3.10a and 10b).

In view of the extensive nature of the coronary artery disease, the large ventricular dyskinetic area and the presence of angina, the patient was discouraged from continuing to run long distance races. Despite this advice, he completed numerous distance races, including the 1978 and 1979 Comrades Marathons both in $10\frac{3}{4}$ hours.

The patient remained well and was without additional cardiac symptoms until August 1979 when, 1 week after completing a standard 42 km marathon in 3 hrs 57 min, he began to experience severe angina particularly when competing in a weekly 16 km time trial. He was initially treated with isosorbide dinitrate 5 mg sublingually. However, after one such race, the patient reported that he had had extreme chest pain and had nearly lost consciousness. Because of this, and in view of his insistence to keep running, he was admitted to Wentworth Hospital, Durban for further cardiological investigations. On clinical examination, there were no significant cardiovascular findings. The patient was not in cardiac

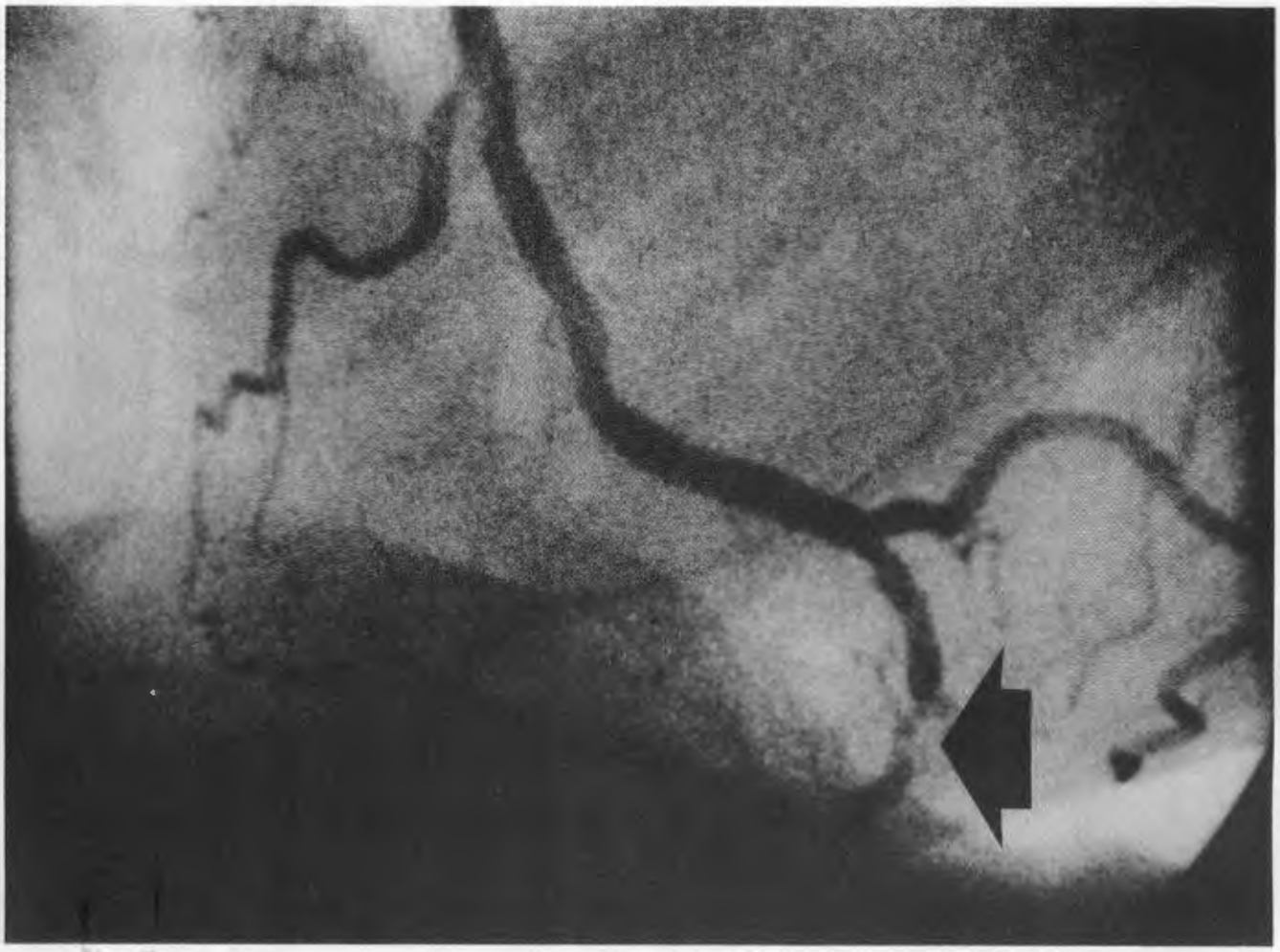

Figure 3.8

Case 4. Right coronary artery in left anterior oblique projection showing mild proximal disease and a 70% area of narrowing in the posterior descending artery (arrow).

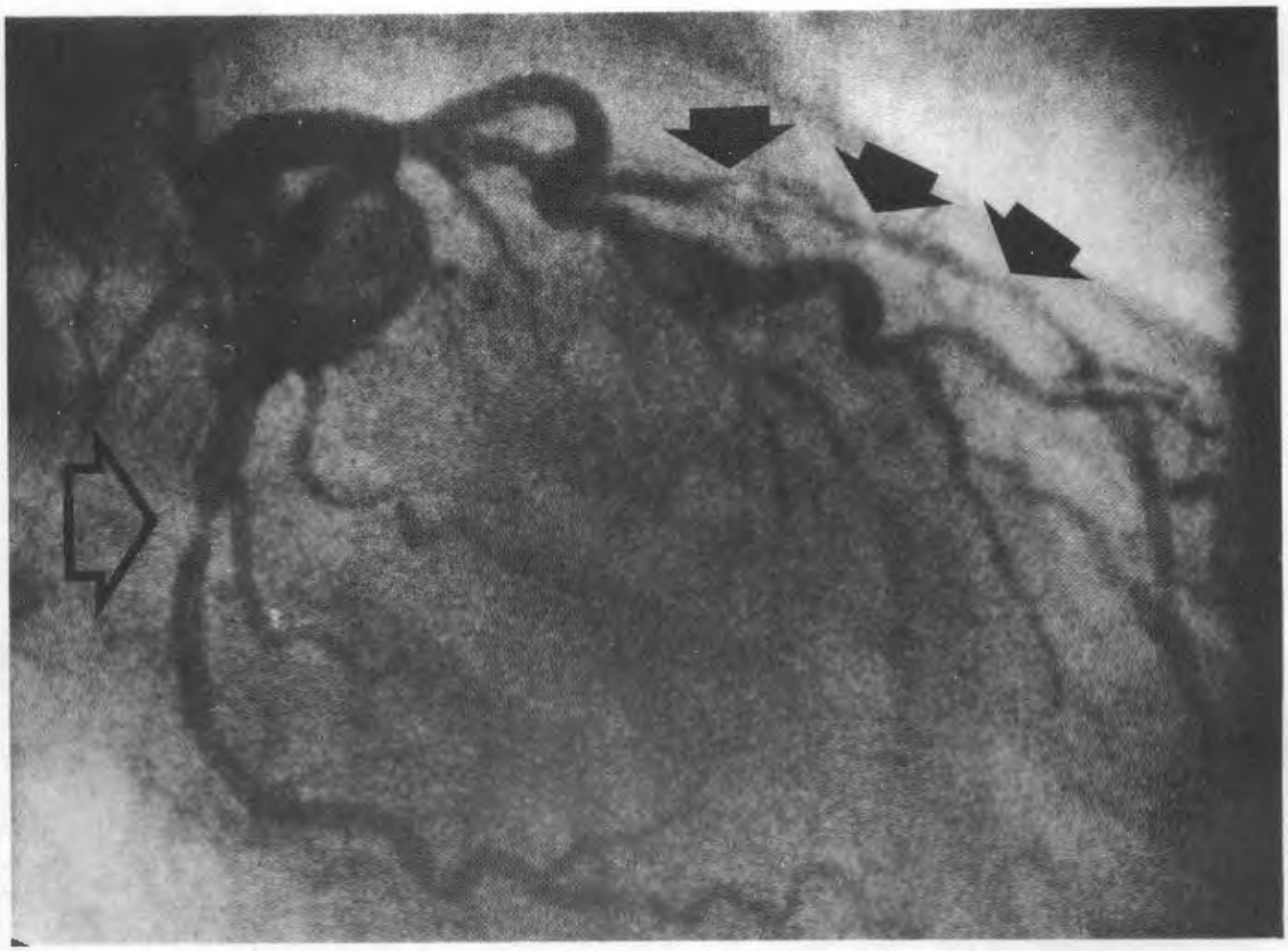

Figure 3.9

Case 4. Left coronary artery in right anterior oblique projection showing an area of stenosis in the circumflex branch (open arrow). The anterior descending coronary artery is narrowed by approximately 80% in at least 3 areas (black arrows).

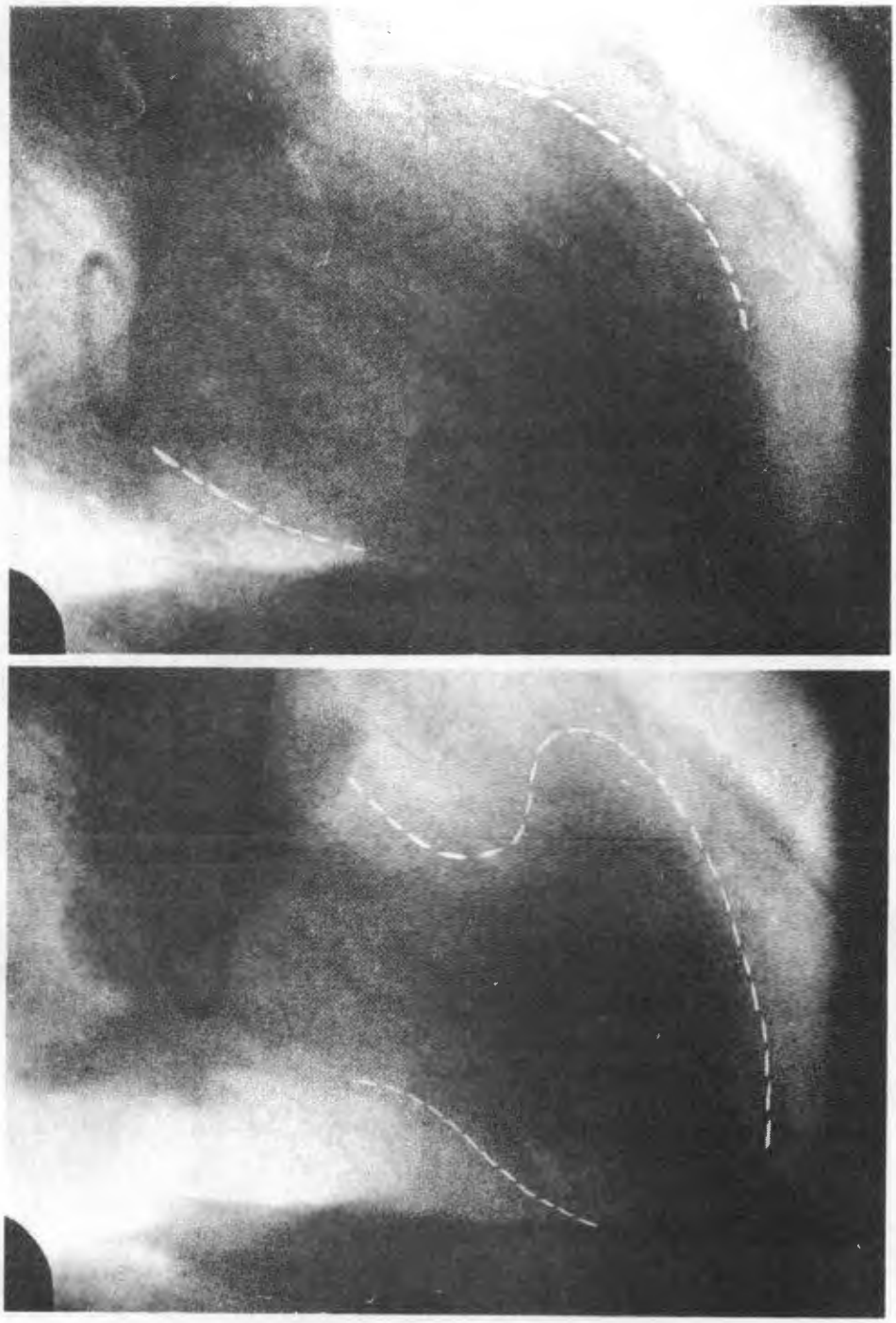

Figure 3 .10

Top. Case 4. Ventriculogram during diastole.

Bottom. Case 4. Ventriculogram during systole showing a large apical akinetic area. Part of the apex and inferior surface of the ventricle has been outlined in white.

failure, and the chest X-ray was normal. The electrocardiogram (Fig. 3.11) showed the old anteroseptal infarction but, in addition, there were persistent ST segment elevations in the anterolateral leads and Q waves in leads 2, 3 and AVF, indicating that an inferior transmural myocardial infarction had occurred since the last electrocardiogram was recorded in December 1976.

Cardiac catheterization revealed the following: the main stem of the left coronary artery, which in 1976 had been free of disease, now showed significant stenosis involving the origins of both the circumflex and left anterior descending coronary arteries; the circumflex coronary artery which previously had only mild diffuse disease now also showed a significant proximal stenosis, whilst the left anterior descending coronary artery was totally occluded (progression from 80% occlusion in at least three areas in 1976), but filled from distal collaterals; the large diagonal branch, shown to be disease-free in 1976, now had a significant stenosis at its origin; the right coronary artery which, in 1976 showed a 70% area of narrowing in the posterior descending branch, now was totally occluded distal to its ventricular branch; and left ventricular wall motion, as assessed by ventriculography, was unchanged from 1976.

In view of the patient's severe disability and the favourable condition of the distal coronary arteries, the patient was referred in January 1980 for coronary bypass grafting to the four diseased coronary vessels. There were no operative or post-operative complications and the patient was discharged home 7 days after surgery. However, 4 days after hospital discharge, the patient developed severe palpitations and on re-admission to hospital was found to be in atrial fibrillation with a ventricular response of 150 beats/min. Sinus rhythm was restored with direct current synchronized conversion and the patient was maintained on

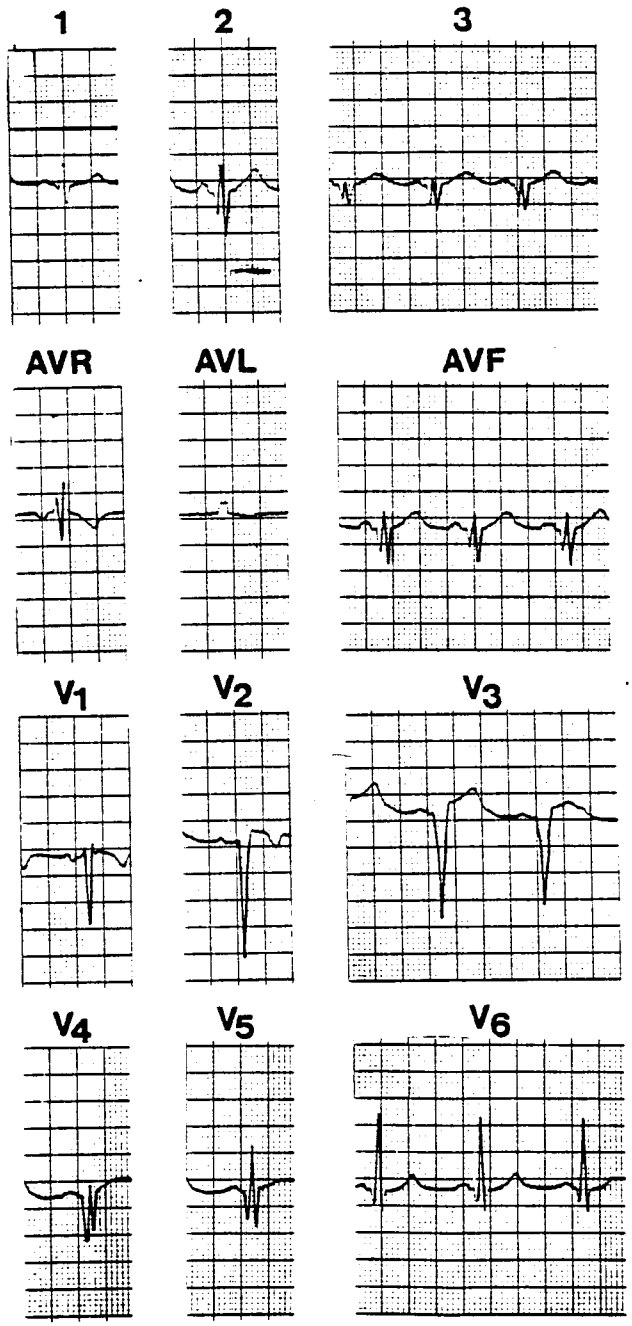

Figure 3.11

Case 4. Resting electrocardiogram taken in November 1979, showing Q waves in leads 2, 3 and AVF and ST-segment elevations in $V_1 - V_4$, changes that were not previously present (Figure 3.7) Certain tracings in this electrocardiogram have been enhanced.

digoxin 0,25 mg daily and verapamil 80 mg 8-hourly, on which medication normal sinus rhythm was maintained. Four days later, the patient was discharged and the verapamil discontinued.

During the 10-month period since his discharge from hospital, the patient has recommenced jogging up to 160 km a month and is on no medication. He is currently asymptomatic, has completed a 28 km race, and plans to run the 1981 Comrades Marathon.

CASE 5.

When first seen in December 1978, this patient aged 55 years had been running for 20 years. During this period he had completed thirteen 90 km Comrades Marathons in times ranging between 9 and 11 hours, more than 100 standard 42 km marathons, and had run in total an estimated 80 000 km.

He had first become aware of symptoms in the 1978 Comrades Marathon, during the first 16 km of which he had experienced mild central chest pain and inappropriate dyspnoea when running uphill. After walking for 3 km he had again been able to run without symptoms and had completed the race "feeling fine". After the race he chose to rest for 2 months before recommencing regular training. Subsequently, during the first kilometer of each run he experienced constricting central chest pain, associated with dyspnoea. Despite this, he was able to run 40 km a week in training.

At the end of November 1978, he entered a 16 km race but half-way through the race, he developed severe, crushing anterior chest pain. He tried unsuccessfully to "run through the pain" but had been forced to walk to the finish line, where he collapsed. Later he consulted a general practitioner, who diagnozed that he had angina pectoris and ordered him to rest. However, in the following week, he developed marked

dyspnoea progressing to orthopnea, pleuritic left-sided chest pain and a non-productive cough which was worse at night. The patient was referred to a physician who found that on clinical examination the heart rate was 40 beats/min, the blood pressure 120/80 mmHg and the jugular venous pressure was raised 8-10 cm above the manubrium sterni. Auscultation of the heart revealed no abnormality but there were crepitations at both lung bases. The electrocardiogram showed Q waves in Leads I, II, AVL, V4, V5 and V6 with T wave inversions in Leads 2, 3 and AVF, V4, V5 and V6. Poor R wave progression was also noted across the anterior chest leads. Figure 3.12 is a representative electrocardiogram taken 3 months later.

The patient was hospitalized and treated with digitalis and a diuretic. The clinical signs of congestive cardiac failure improved, but on the 10th day in hospital the patient developed pleuritic chest pain and haemoptysis, but without radiological changes in the chest. This was diagnosed clinically as a pulmonary infarction. The patient was discharged on the 30th day after admission.

For 3 months after discharge, the patient experienced dyspnoea even on minimal exertion, associated not with classical angina but with a feeling of "fullness" in the chest. In view of the severe degree of the patient's disability, he was referred, in May 1979, to Wentworth Hospital, Durban for cardiac evaluation.

The relevant features of the patient's past medical history were that 7 years previously he had been diagnosed as a "mild" hypertensive and had since that time been treated with Brinerdin, 1 tablet daily. This treatment had controlled his blood pressure at values between 120 and 140 mmHg systolic. It should be noted that the patient received normal life insurance at age 22 years, indicating that his hypertension was of more recent origin. The patient's father had died from a heart attack at the

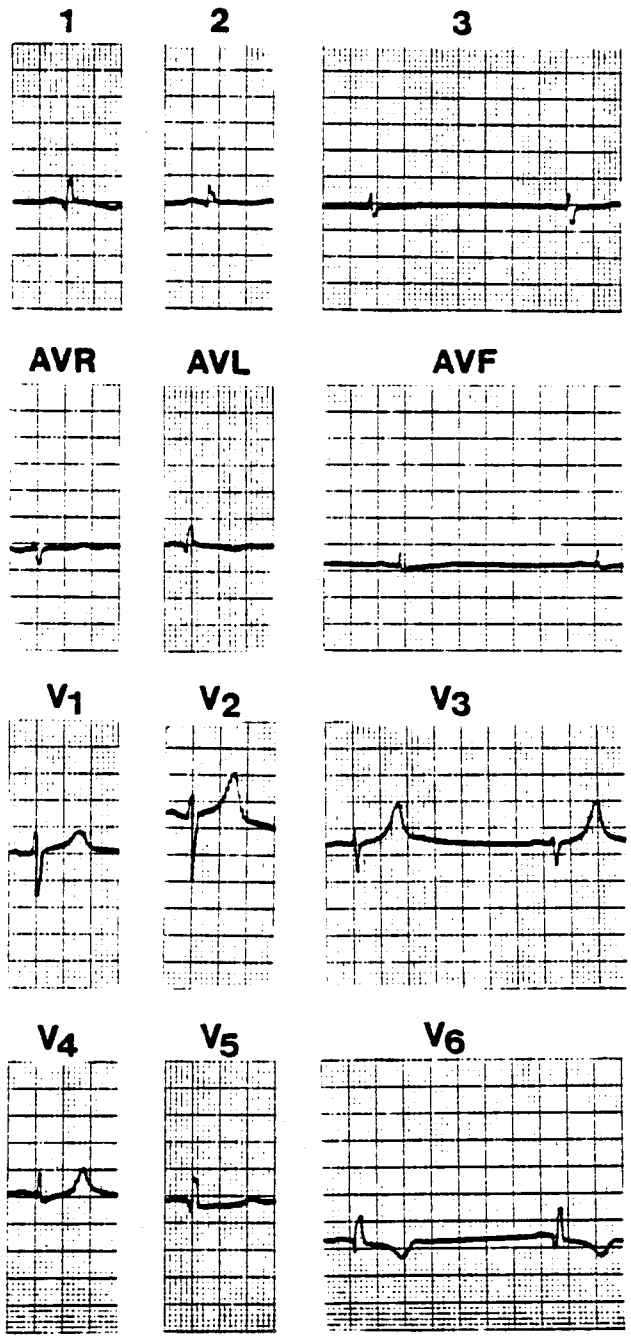

Figure 3.12

Case 5. Resting electrocardiogram showing Q waves in leads 1, 2, V_5 and V_6, and poor R wave progression in the anterior chest leads.

age of 55 and his brother suffered an acute myocardial infarction at 58, but had recovered and is at present well. The patient had been a moderate smoker (30 cigarettes a day) from ages 21 to 36 but had stopped completely when he started running. He drinks approximately 3 ounces of alcohol per week.

On cardiovascular examination, the heart rate was 64 beats/min with occasional irregular beats, and the blood pressure was 180/110 mmHg. The jugular venous pressure was not raised and there were no abnormal cardiac sounds. The lung fields were clear and,on ophthalmoscopy, the retinal blood vessels appeared normal. The electrocardiogram was unchanged from that shown in Fig. 3.12 and was considered compatible with old anterolateral myocardial infarction with apical aneurysm formation. The chest Xray showed a pseudo boot-shaped heart with an increased cardio-thoracic ratio of 55%.

Special investigations showed normal haematological, serum electrolyte and serum enzyme patterns. However,serum uric acid was elevated at 0,61 mmol/L (normal values 0,15-0,42). Serum cholesterol was 6,6 mmol/L (normal values 3,9 - 6,5 mmol/L) with an HDL of 1,11 mmol/L, giving a total cholesterol:HDL cholesterol ratio of 5:9. The serum triglyceride level was 1,68 mmol/L (normal values 0,30-1,81 mmol/L) and no chylomicrons were observed in the fasting serum.

Prior to coronary angiography, the patient's blood pressure was stabilized at 140/85 mmHg on furosemide 40 mg daily, prazosin 2 mg 6-hourly, and supplemental potassium.

Coronary angiography showed a 50% narrowing in the left mainstem coronary artery immediately proximal to its bifurcation (Fig. 3.13). The left anterior descending coronary artery was significantly narrowed in three areas, whereas the circumflex coronary artery showed complete occlusion of

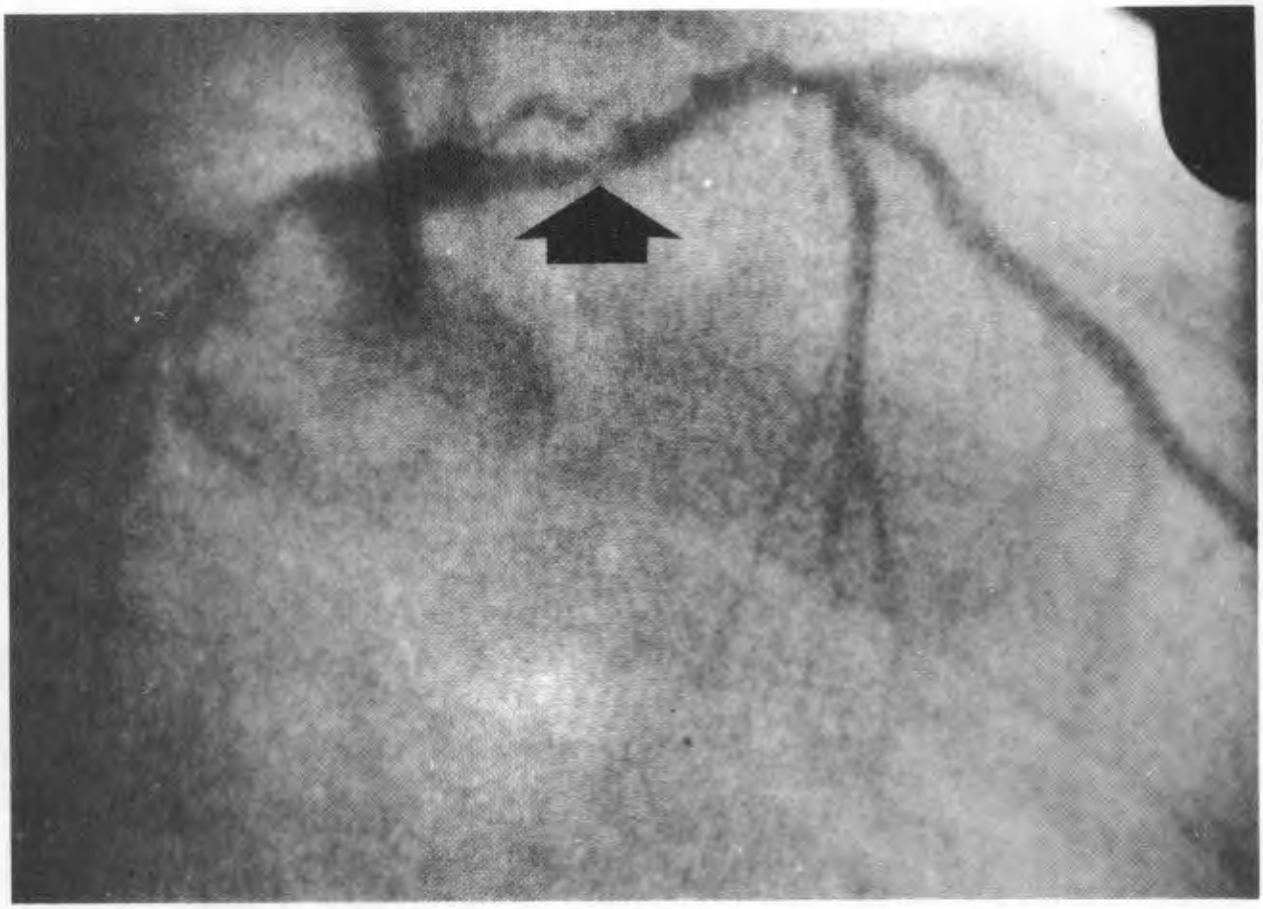

Figure 3·13

Case 5. Left coronary artery in right anterior oblique projection showing 50% narrowing in the left main-stem proximal to its bifurcation (arrow).
The diseased circumflex coronary artery is poorly visualized in this reproduction.

its proximal branch, the ramus medialis, and the distal artery was small and diffusely narrowed. The right coronary artery was totally occluded in its proximal third and the distal vessel filled from the dominant left coronary artery (Fig. 3.14). Ventriculography showed a dilated left ventricle with an anterolateral aneurysm and a hypokinetically-contracting inferior surface (Fig. 3.15a and b). Because of the poor state of the patient's distal coronary arteries, coronary bypass surgery was not considered to be feasible, and the patient was discharged from hospital on medication to participate in a mild exercise rehabilitation programme.

C. Relevance of these cases to the "Bassler Hypothesis".

The cases described above indicate that myocardial infarction, and angiographically-proven coronary artery disease, indistinguishable from coronary atherosclerosis, can occur in marathon runners. Furthermore, despite their being marathon runners, each for at least 10 years, cases 3, 4 and 5 all had coronary artery disease which, in the latter 2 cases, was incapacitating. Nor were any of these 3 runners smokers, nor did they have abnormal blood lipid levels or long-standing hypertension.

Thus, these cases show that marathon running cannot guarantee absolute protection against either myocardial infarction or angiographically proven coronary atherosclerosis, even in the absence of the 3 major risk factors - smoking, hypertension and hypercholesterolaemia.

Further points arising from these cases are reviewed in the concluding summary of this chapter.

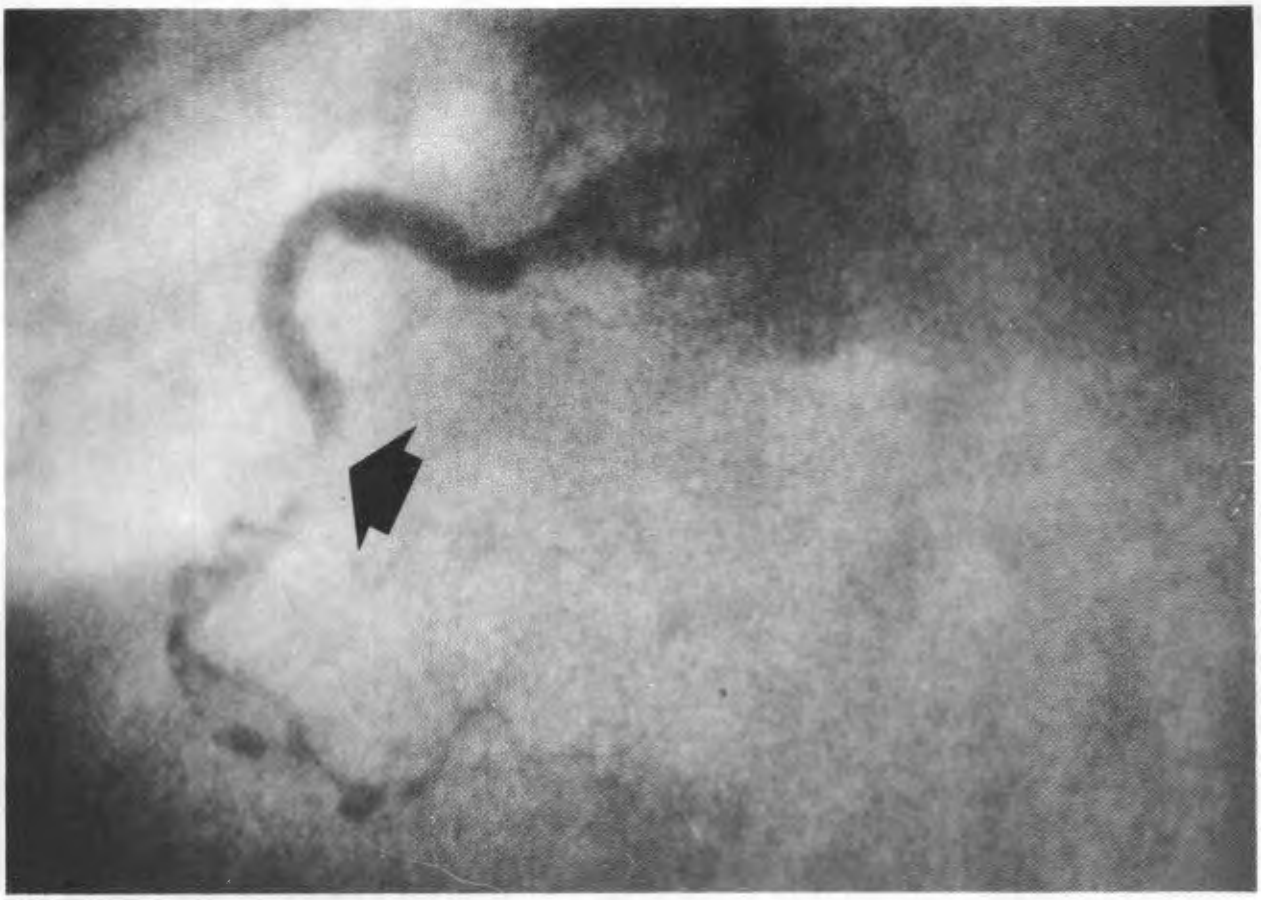

Figure 3·14

Case 5. Right coronary artery in anterior oblique projection showing complete occlusion in the proximal third of the artery (arrow).
The distal vessel filling from the left coronary artery.

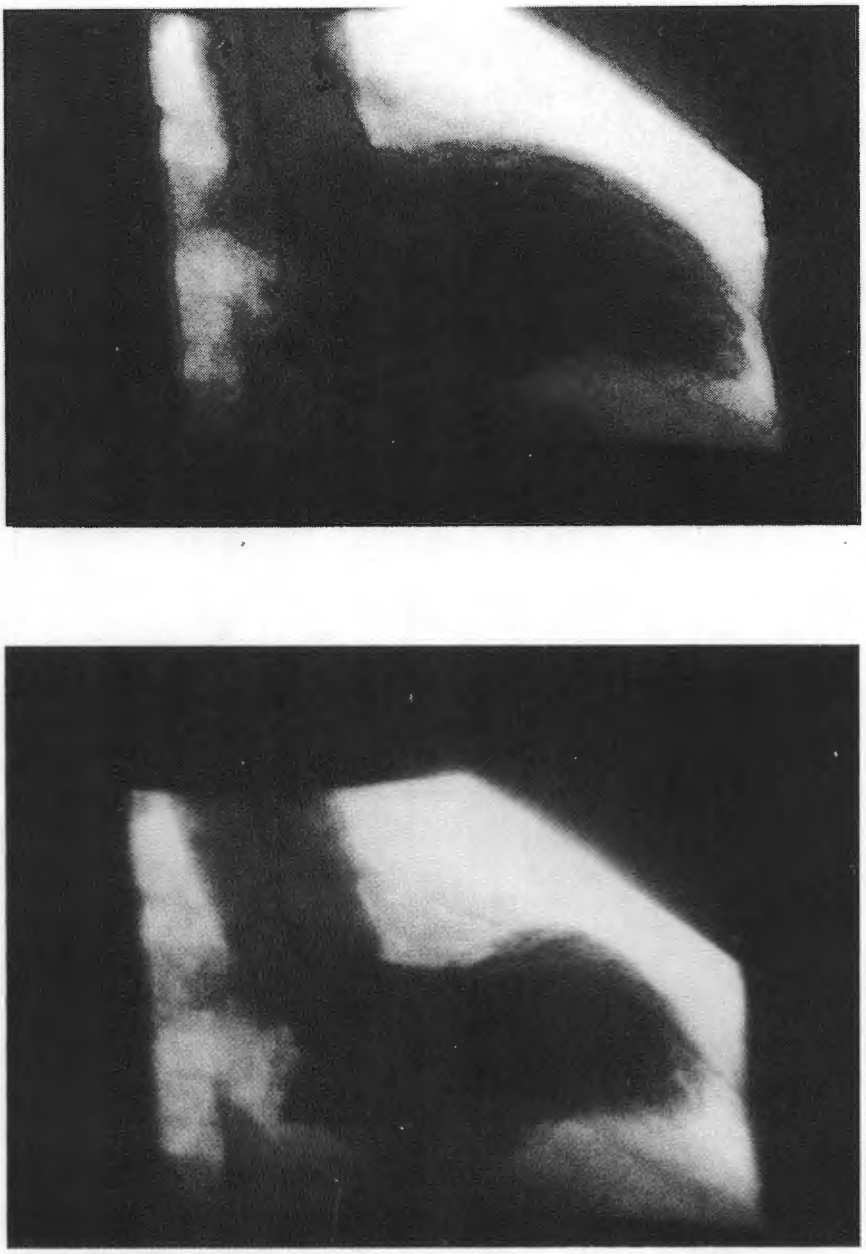

Figure 3.15

Top. Case 5. Ventriculogram during diastole.

Bottom. Case 5. Ventriculogram during systole showing anterolateral aneurysm and poorly contracting inferior surface.

3.3 SUDDEN DEATH IN MARATHON RUNNERS.

A. Cases in which coronary atherosclerosis was shown at autopsy.

CASE 6. (Reference 96 and 394)

A white male aged 39 in 1976, participated in rugby football and amateur boxing in which he was nationally ranked until the age of 18, after which he became physically inactive. Apart from occasional acute attacks of gout from 1963 onwards, for which he took allopurinol, he had no cause to seek medical attention. In January 1974 he took up long distance running and in the course of the following 27 months completed two 90 km Comrades Marathons, a number of standard marathons, and had run 6 480 km in training. By the end of March 1976, he was in full training for that year's Comrades Marathon and was running more miles at a faster pace than at any previous time in his life. However, having completed his usual Sunday morning run of 42 km uneventfully, he became aware of a sharp stabbing pain in his right elbow shortly after commencing a training run the following Monday, March 28. The pain persisted and after a further 3 km was followed by the onset of moderately severe, cramping, anterior chest pain. These pains became progressively worse until he was forced to walk. Within a minute of walking the chest pain disappeared only to reappear immediately he started jogging. The patient returned home and although he felt sluggish he had no further chest pains. On the following 2 days the patient walked and ran a total of 30 km and was again troubled by the same pains. But on Thursday morning, he was awakened by severe, crushing precordial chest pain radiating to the right elbow and associated with

sweating and nausea.

He went immediately to hospital where the electrocardiogram showed a hyperacute inferolateral myocardial infarction with marked ST-segment elevation in leads 2, 3, AVF, V5 and V6 with pathological Q waves in 3, AVF and V6. On admission to the Intensive Coronary Care Unit, the only abnormal findings were a jugular venous pressure raised 2 cm above the manubrium sterni and a third heart sound. He was treated with digoxin, furosemide, and supplemental potassium for mild right ventricular failure.

Cardiac enzymes were elevated the day after admission (creatine phosphokinase-510 units/ml, normal up to 50; lactate dehydrogenase - 283 units/ml, normal up to 90),but returned to normal values within 4 days. The patient was discharged from hospital on propranolol 40 mg 8-hourly, and on sulphinpyrazone. On the day of his hospital discharge he walked half a mile without chest pain and returned to work the following morning, 12 days after acute myocardial infarction.

Over the next 3 weeks he increased his daily walking distance to 3 km,at which stage he resumed jogging. For a period he developed elbow and chest pain when jogging, but always walked immediately when any chest pain started. By the end of June he was jogging without symptoms up to a distance of 6 km, and in mid-July 1976 he was admitted to Groote Schuur Hospital for assessment of his cardiovascular status.

There was no family history of heart disease or hypertension, but one maternal aunt had died from diabetes in her forties. The patient did not drink alcohol but had smoked three pipefuls of tobacco and 2 to 3 cigarettes a day, from the age of 18, until one month after his myocardial infarction.

The patient was small (5'6"), muscular white male (somatotype

2:6½:1 Heath-Carter) with 13,6% body fat estimated from skinfold measurements. There were no stigmata of hyperlipidemia. The patient's blood pressure was normal (110/70 mmHg) and the resting heart rate was 50 beats/min. The apex beat was not displaced, there were no abnormal ausculatory findings, and all the peripheral pulses were present. Other features, including urine and blood count,were normal and the ESR was 5 mm/hr (Westergren). The glucose tolerance test was normal, serum uric acid was elevated (> 12 mg/100 ml) and the fasting lipogram was classified as a type IIb hyperlipidemia with cholesterol 265 mg/100 ml, triglycerides 235 mg/100 ml (normal up to 150), chylomicrons 16 mg/100ml (normal up to 12), pre-β-lipoproteins 177 mg/100 ml (normal up to 150), and β-lipoproteins 513 mg/100 ml (normal up to 48).

The chest Xray was normal and a resting electrocardiogram showed an old inferior myocardial infarction (Fig. 3.16). The effort electrocardiogram did not reveal any symptoms, arrhythmias or ST segment changes up to a heart rate of 130 beats/min while on propranolol 40 mg twice daily. The echocardiogram showed normal sizes and movement of heart chambers,and there was no thickening of the posterior ventricular wall. The patient was Holter-monitored during a 30-minute jog, during which time no arrhythmias were recorded.

Left heart catheterization showed an increased left ventricular end-diastolic pressure (20 mmHg). Angiography revealed complete occlusion of the distal circumflex coronary artery just after the origin of the obtuse marginal branch (Fig. 3.17), with a small segment of the more distal vessel filling later by means of bridge collateral. The left anterior descending coronary artery had minor luminal irregularities (Fig. 3.17). There was a 50% narrowing of the right coronary artery, which involved the origin of the right ventricular branch. The rest of the vessel was of good calibre with

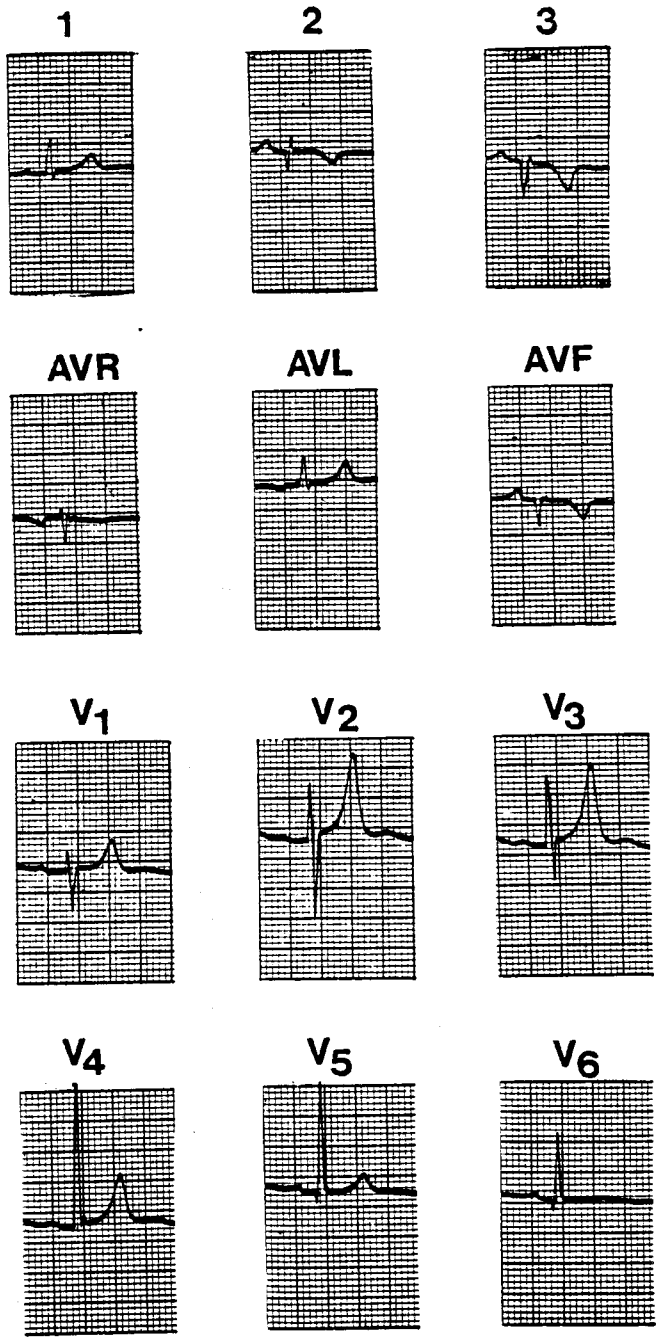

Figure 3.16

Case 6. Resting electrocardiogram showing Q waves in leads 2, 3 and AVF. The tall T waves in V_2 and V_3 are normal for athletes. (References 278, 279, 288).

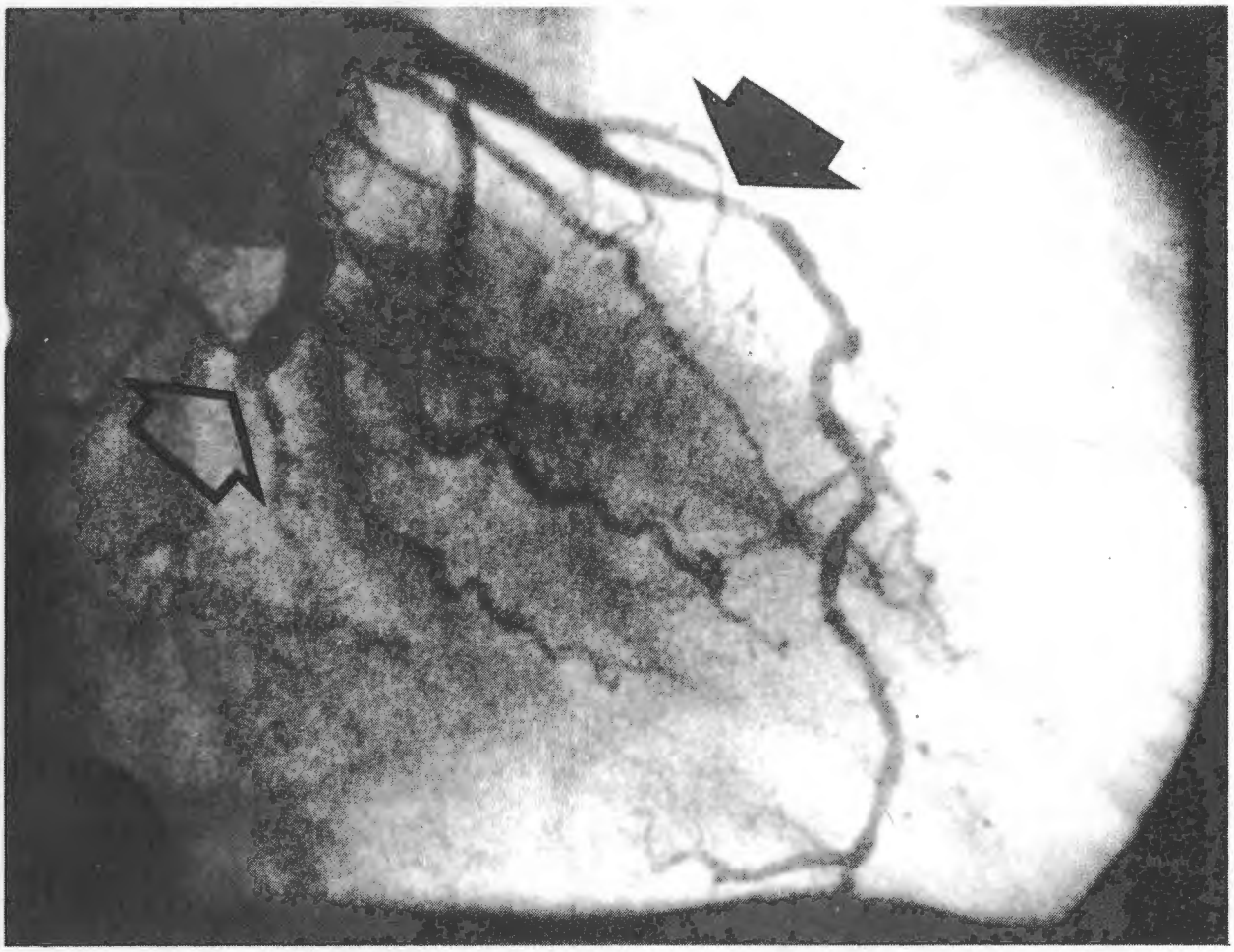

Figure 3.17

Case 6. Left coronary artery in right anterior oblique projection showing complete occlusion of the circumflex artery (open arrow) and luminal irregularities in the anterior descending coronary artery (black arrow).

some luminal irregularities (Fig. 3.18). Cineangiography of the left ventricle showed good contraction except for an area of inferior and infero-basal hypokinesia (Fig. 3.19).

The patient was treated with propranolol 40 mg twice daily and clofibrate. For exercise rehabilitation, he was advised to continue jogging but not to participate in marathon races.

Despite this advice, he gradually increased his training and on 5 March 1977, approximately one year after myocardial infarction, he completed a 50 km race in 5 hours 36 min, and 2 weeks later a 42 km marathon in 4 hours 45 min. He ran additional 42 km marathons in April and September 1977, and August 1978, the latter in a time of 4 hours 5 min. In the 28-month period between his myocardial infarction and this latter race, he had run 3624 km and completed 5 marathons as documented in his running logbook subsequently made available to us. The logbook also records numerous episodes of pain in the chest or jaws or left arm, during training runs, but he did not seek medical advice for these complaints.

In September 1978 he was re-admitted to hospital with a history of two attacks of constricting central chest pain lasting for 30 and 45 minutes, radiating to the jaw and to both elbows and not responding to nitroglycerin. His pulse rate was 56 beats/min and blood pressure 150/80 mmHg. Serum creatine kinase was slightly elevated (114 iu/L; normal up to 70) and the only electrocardiographic change was transient T wave flattening in leads 1 and V4 and V6. The patient was treated for unstable angina with propranolol 40 mg 8-hourly and isosorbide dinitrate 10 mg 2-hourly. The chest pain settled and he was discharged 6 days later.

However, on the following day, he again developed chest pain lasting a few hours for which he was re-admitted to hospital. There were no fresh electrocardiographic changes but the serum creatine kinase was again mildly elevated (133 iu/L). As the pulmonary wedge pressure measured

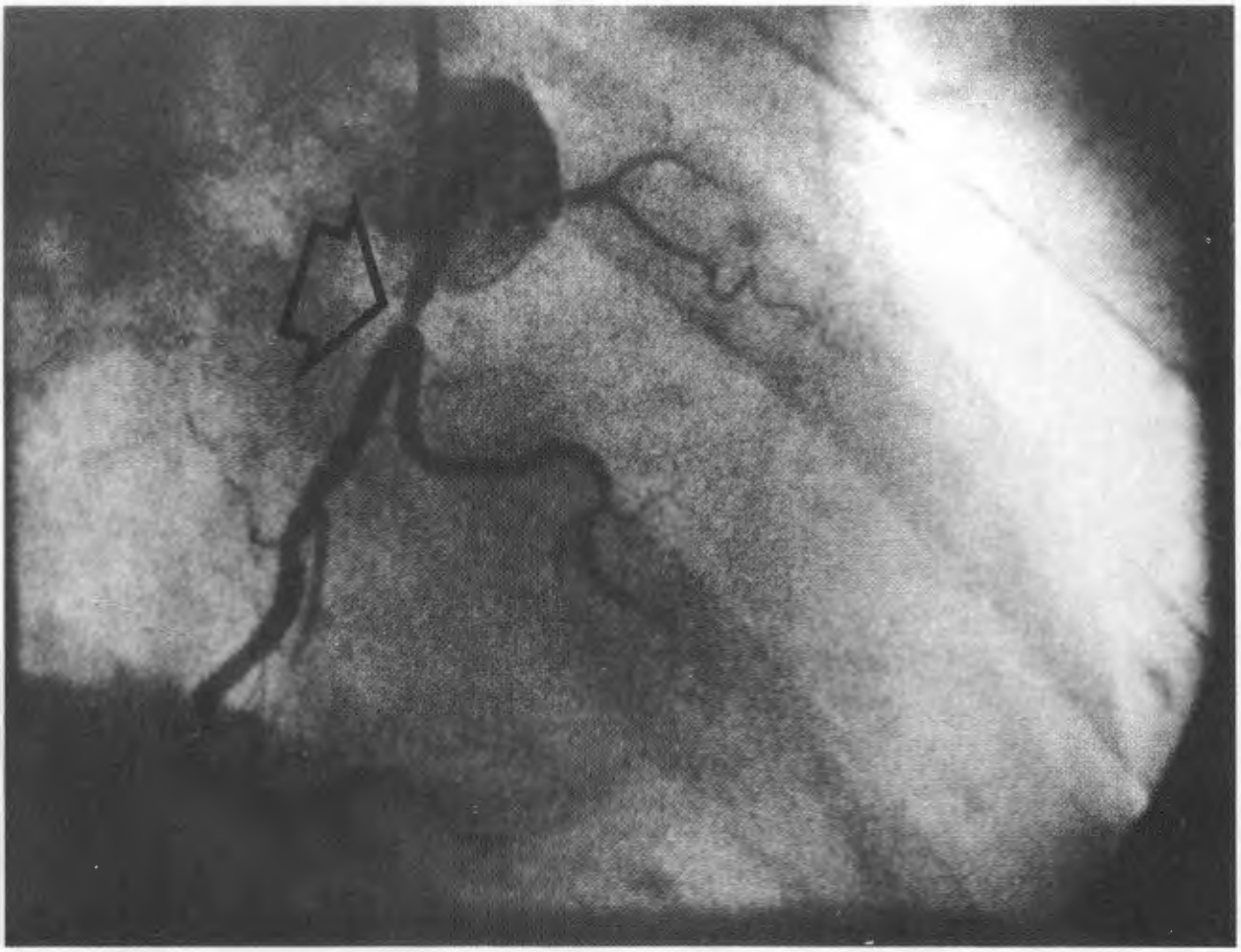

Figure 3.18

Case 6. Right coronary artery showing 50% proximal narrowing (arrow).

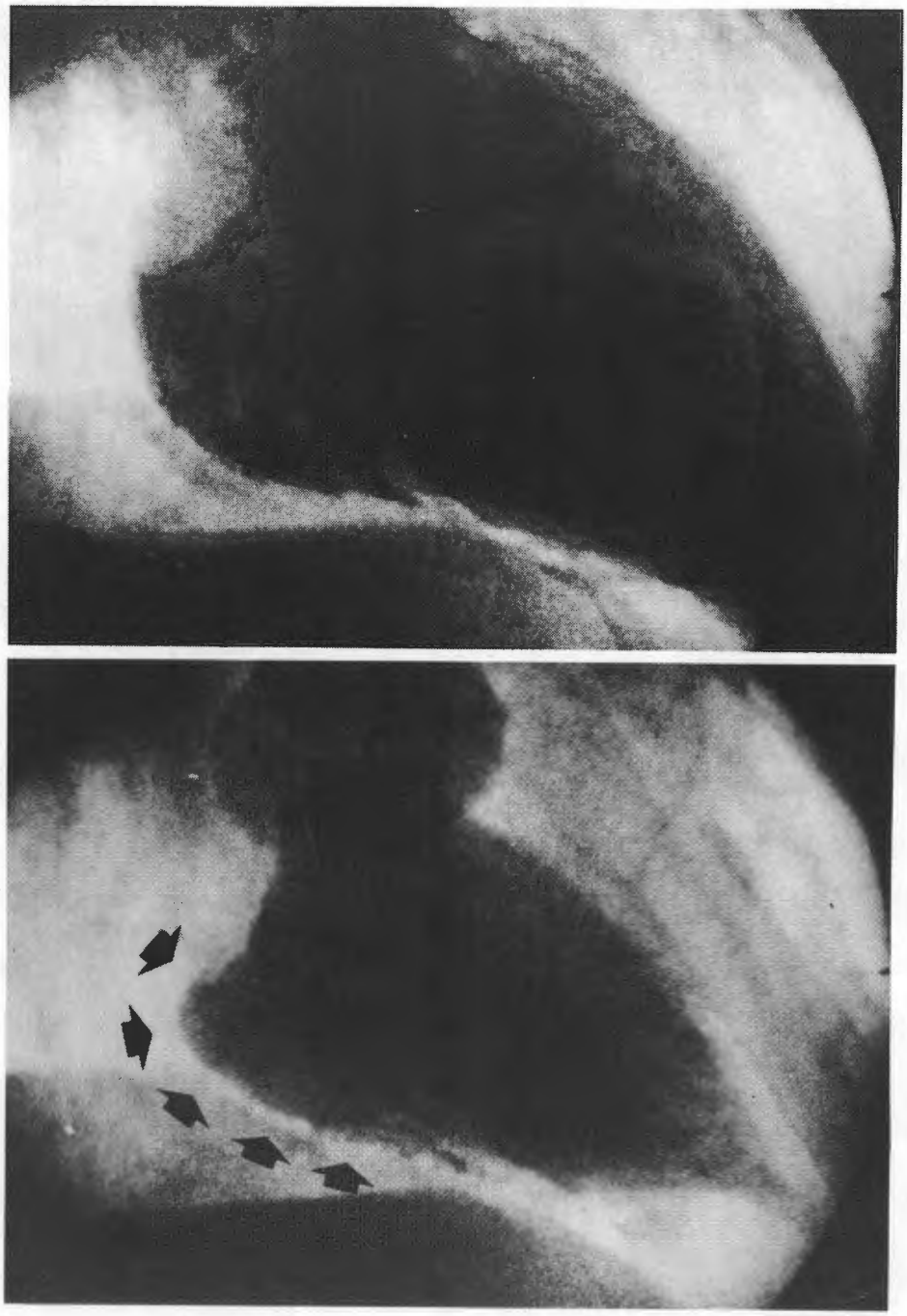

Figure 3.19

Top. Case 5. Ventriculogram during diastole.

Bottom. Case 5. Ventriculogram during systole showing inferior and infero-basal hypokinesis (arrows).

by a Swan-Ganz catheter was normal (8 mmHg), the dose of propranolol was increased to 60 mg 8-hourly. He was discharged from hospital 1 week later.

In October 1978 a repeat coronary angiogram showed complete occlusion of the circumflex coronary artery (as noted before), total occlusion of the right coronary artery (progression from a 50% lesion found in 1976) and greater than 80% stenosis of the anterior descending coronary artery (progression from minor luminal irregularities found in 1976).

On 14 October, whilst working in his garden, he developed severe chest pain and was re-admitted to hospital. Serum creatine kinase was normal (27 iu/L) and the electrocardiogram had only T wave abnormalities in V4 and V6. But on the evening of 17 October, whilst awaiting coronary bypass surgery, he began to have severe chest pain and electrocardiography revealed acute anterolateral ST-segment elevation with increasing size of the Q waves in V5 and V6 (Fig. 3.20). Despite therapy, the heart stopped within 1½ hours and resuscitation failed. Consent was given for only a partial post-mortem examination undertaken 58 hours after death.

The heart weighed 344 grams. Drill-biopsy specimens were taken from the macroscopically normal left ventricular free wall and septum for the analysis of potassium/sodium ratios[400]. The three major epicardial coronary arteries were sectioned transversely at 3 mm intervals throughout their length and all the transverse sections so obtained were processed and examined microscopically. The degree of luminal narrowing in these vessels is indicated in graph form (Fig. 3.21). The mainstem of the left coronary artery was free of significant disease, but its anterior descending branch showed up to Grade 4 (75-100%) narrowing. Total occlusion by a fresh thrombus superimposed on grade 4 atherosclerotic luminal narrowing was seen 50 mm from its origin (Fig. 3.22). In the left circumflex artery there was up to Grade 4 narrowing, and at 40 mm from its origin the vessel was totally

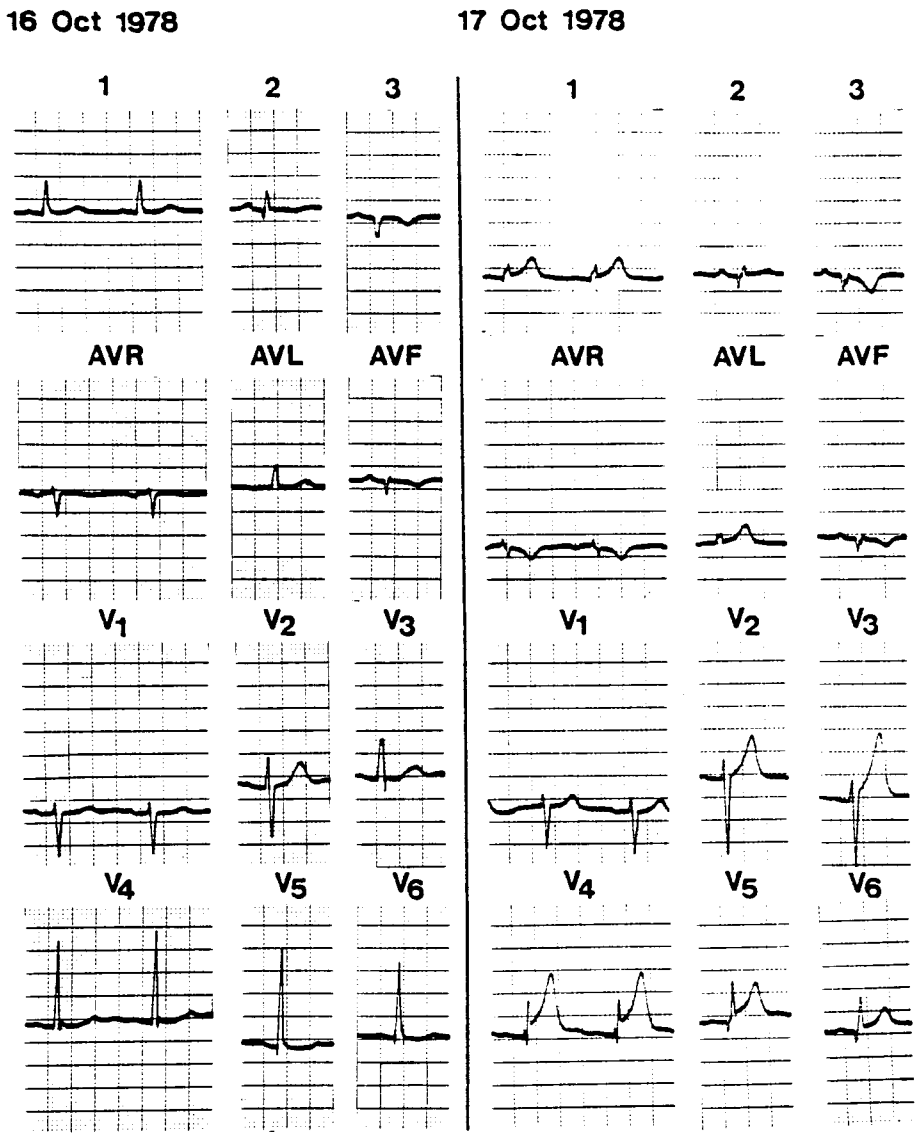

Figure 3.20

Case 6. Resting electrocardiograms taken 1 day apart. The latter tracing taken shortly after the onset of chest pain shows antero-lateral ST-segment elevation and increasing size of the Q waves in V_5 and V_6.

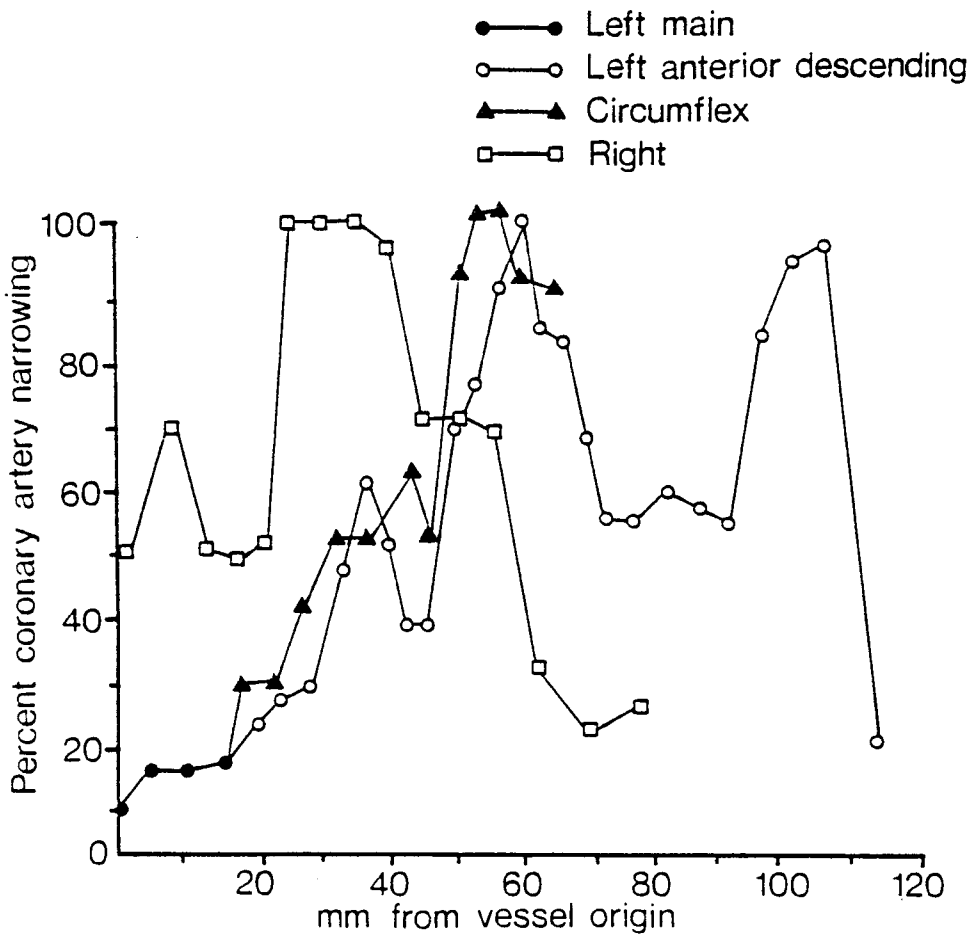

Figure 3.21

Case 6. Graph showing percentage luminal narrowing of three major epicardial coronary arteries obtained by histological examination of repetitive transverse sections 3mm apart. Each artery is total occluded in at least one site.

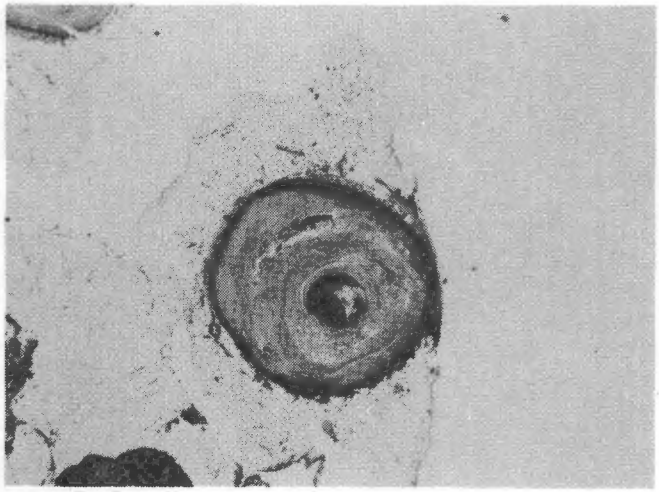

Figure 3.22

Case 6. Anterior descending coronary artery showing total occlusion by a fresh thrombus superimposed on Grade 4 (75-100%) atherosclerotic luminal narrowing 5cm from its origin.

occluded by long-standing organized thrombus. In the right coronary artery there also was up to Grade 4 narrowing,and 23 mm from its origin the vessel was totally occluded for 6 mm by a recently organized thrombus.

The healed infarct of the left ventricular inferior wall was composed of mature, hypocellular fibrous tissue. Multiple dilated thin-walled blood vessels were present,but there were no inflammatory cells. These appearances were in keeping with a healed infarction of over 2 years duration. Incubation of a transverse slice of the heart through both ventricles with nitroblue tetrazolium[401] showed deficient enzyme activity only in the scarred wall of the left ventricle. Although there were neither histologic nor enzymatic changes of fresh infarction, acute myocardial infarction was strongly suggested by the clinical features, the recent thrombus, and by the depressed potassium/sodium ratios[402] found in the left ventricular free wall and septum (0,96 $\pm$ 0,08; mean $\pm$ SEM for 9 biopsies). Biopsies were taken by the drill biopsy technique of Pool, Norris, Lewis and Covell[403] and analyzed for myocardial potassium/sodium ratios by the method of Jennings, Crout and Smetters[404].

The sino-atrial node was not present in the heart specimen submitted. The atrioventricular node showed mild interstitial fibrosis but the bundle of His was normal. The descending thoracic aorta showed moderate atherosclerosis, and lung section revealed acute oedema.

CASE 7.

A 44 year old man had been running long-distance events for 14 months. During this period he completed eight standard 42 km marathons, a 56 km race in 5 hours 59 minutes, and, on May 31 1978, the 90 km Comrades Marathon in 10 hours 10 minutes. In training, he ran from 48 to 80 km per week. He was a non-smoker and a social drinker, and there was no relevant

history of serious disease. In 1970 he received life insurance from two companies at a normal premium, indicating the absence of any apparent cardiovascular abnormality.

In July 1978, he ran a 50 km race in 4 hours 59 minutes, and on August 13, 1978, a standard 42 km marathon in 4 hours 2 minutes. At about this time, he visited a general practitioner and complained of a non-specific lack of energy. There were no symptoms or signs of ischaemic heart disease. The blood pressure was described as normotensive, although the actual value was not recorded. The fasting serum cholesterol level was 7,65 mmol per litre (296 mg/100 ml), the high-density lipoprotein fraction 0,43 gram per litre (43 mg/100 ml), and the triglyceride level 1,63 mmol per litre (145 mg/100 ml). The blood count and erythrocyte sedimentation rate were normal. Electrocardiography was not performed.

On September 16, 1978, the athlete competed in the 24 km Simba road race in Johannesburg. He was running without distress at the 19 km mark, when he stopped to adjust a loose shoelace. Bending down, he suddenly lost consciousness and fell to the ground. The first runner to reach him noted that he was immobile and pulseless and that facial pallor rapidly developed. All attempts at resuscitation failed.

At autopsy 48 hours after death, there were not extracardiac findings to explain the cause of death. The heart and some skeletal muscle were removed from the body, placed in ice and flown directly to Cape Town for further analysis. Fifty six hours after death, drill biopsy specimens were taken from the macroscopically normal left ventricular epicardium and from the skeletal muscle for analysis of potassium/sodium ratios.

The heart weighed 357 grams and showed extensive anteroseptal infarction (Fig. 3.23). This healed infarction appeared to be of at least 3 to 6 months duration and consisted of a mature scar devoid of inflammatory cells. Sections for microscopical examination were taken at 10 mm intervals

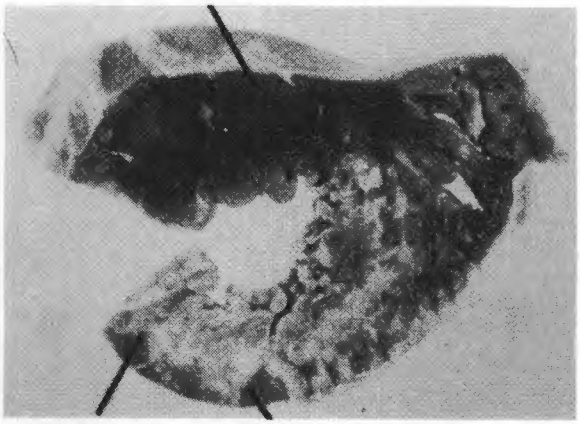

Figure 3.23

Case 7. Transverse slice of both ventricles after incubation with nitroblue tetrazolium. The healed anteroseptal infarction is represented by the white scarred area in the lower portion of the picture. Enzyme staining is lost throughout the entire anterior, and in a small zone of the posterior epicardial, left ventricular walls, and in the right ventricular posterior wall. The potassium-sodium ratios were reduced as indicated.

along the course of the 3 coronary arteries for assessment of luminal narrowing (Fig. 3.24). About 5 mm from its origin the anterior descending branch of the left coronary artery showed Grade 4 (75 to 100%) atherosclerotic narrowing of the lumen (Fig. 3.25) and 15 mm from its origin the artery was occluded by a completely organized thrombus, in which some recanalization had occurred. The extent of atherosclerosis in the circumflex coronary artery is also shown in Fig. 3.25. Light microscopy revealed fibrosis of the papillary muscles. There was no evidence of coagulative necrosis, but in the subendocardium of the left ventricular posterior wall cytoplasmic contraction bands[400,405,406] as well as local fibrosis were observed. In this portion of subendocardium no enzyme activity was detected with nitroblue tetrazolium (Fig. 3.23). Contraction bands were also present in the posterior wall of the right ventricle, which likewise showed no dehydrogenase enzyme activity. The atrioventricular node and the bundle of His were microscopically normal. The sinoatrial node was not included with the heart received for examination.

In biopsy specimens from the macroscopically normal epicardial left ventricular wall, the potassium-sodium ratios were much reduced (0,63 $\pm$ 0,06, mean $\pm$ SEM in 6 specimens) whereas values in the skeletal muscle were normal (2,40 $\pm$ 0,19 in 8 specimens) - a combination found in sudden cardiac death[402].

CASE 8.

A 36 year old man began serious long-distance running in January 1971 and in the 7 years before his death had run approximately 80 km a week. Each year he had competed in 4 or more standard marathon or longer races, including three 90 km Comrades Marathons and a 160 km track race. For the 9 years up to 1969, he had smoked approximately 10 cigarettes a day, but had

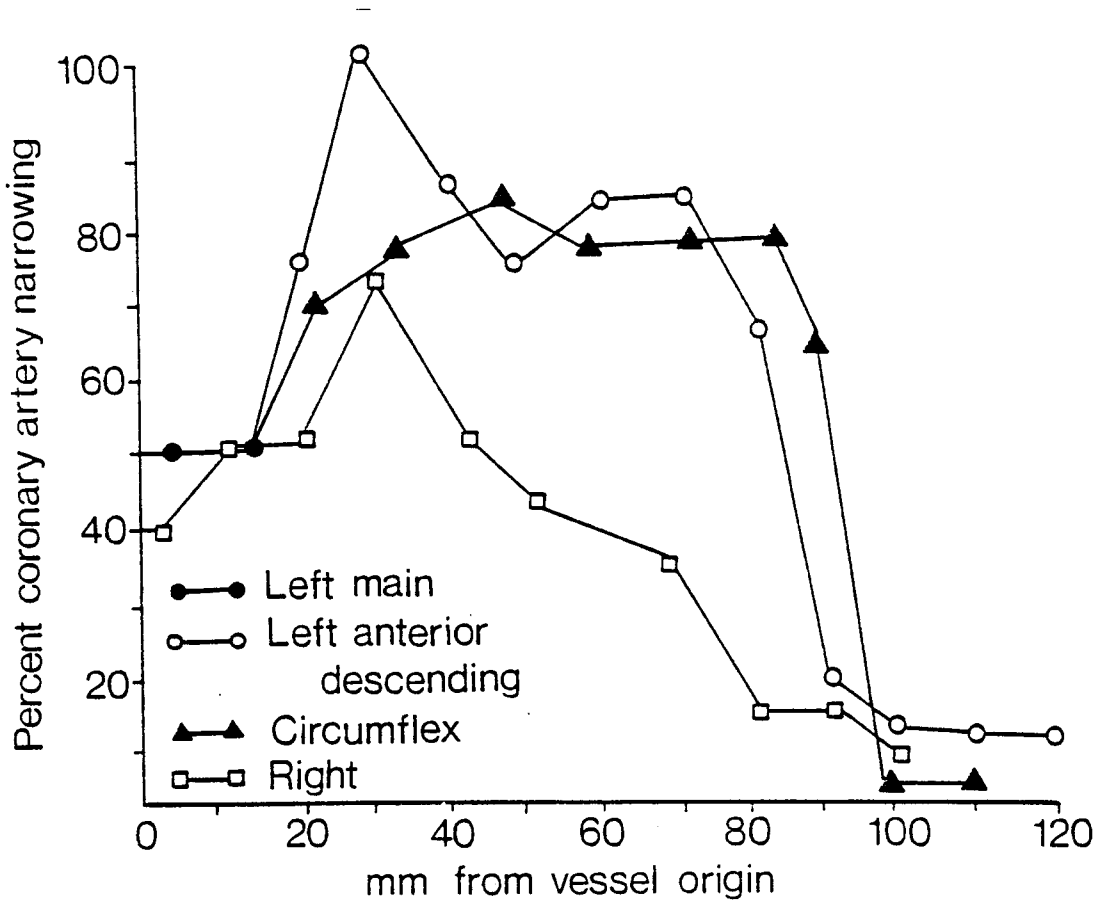

Figure 3.24

Case 7. Graph showing percentage reduction of luminal cross-sectional area in the 3 major coronary arteries obtained by histological examination of repetitive transverse sections 10 mm apart. There was extensive (70 to 80%) narrowing extending for about 60 mm of the circumflex and left anterior descending coronary arteries, and one site of total occlusion of the latter artery.

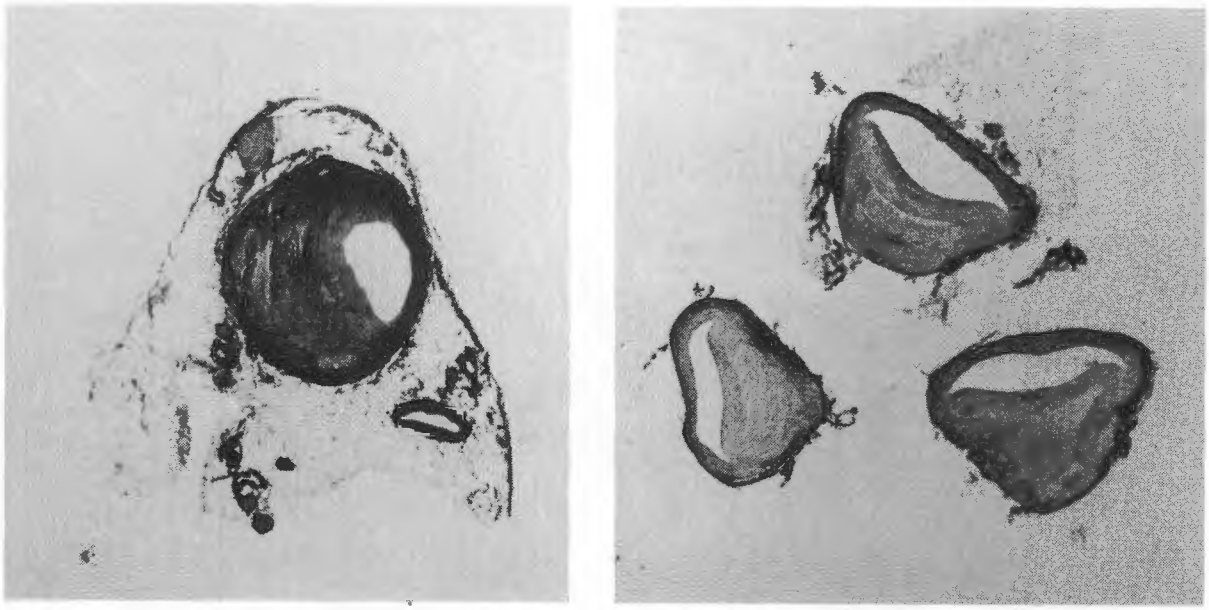

Figure 3.25

Case 7. Anterior descending and circumflex branch of left coronary artery.
In the left panel, the anterior descending branch of the left coronary artery 5 mm from its origin shows Grade 4 (75 to 100%) atherosclerotic narrowing (elastin stain by van Gieson, x 7.5). In the right panel, the left circumflex coronary artery shows Grade 4 (75 to 100%) eccentric atherosclerotic narrowing 3.5 cm from its origin (haematoxylin and eosin stain, x 7.5).

not smoked again after that date. In February 1976, a routine medical examination revealed no symptoms or signs of cardiovascular disease. His blood pressure was 130/80 mmHg and the resting and submaximum-effort electrocardiograms (to a heart rate of 150 beats/min) were both normal. On April 30, 1978, while on a long training run in preparation for the 1978 Comrades Marathon, he and two other runners (one of whom is Case 9) were instantly killed by an automobile. Forensic autopsies were performed.

The heart weighed 360 grams and the myocardium was normal. The anterior descending branch of the left coronary artery (Fig. 3.26) and one of its large diagonal branches (Fig. 3.27) showed advanced luminal narrowing by concentric atherosclerosis of the intima. The left circumflex branch and the right coronary artery showed milder atherosclerotic narrowing. The small coronary vessels were normal. Histologic study of the ventricular myocardium showed no evidence of infarction or fibrosis. The only additional abnormality detected was a localized area of fibrous thickening on the contact area of the anterior mitral leaflet.

CASE 9.

A 27 year old man had been an active athlete at school and had excelled at rugby football and middle-distance running. In February 1977 he began long-distance running and in the following 14 months he completed three 42 km standard marathon or longer races. One week before his accidental death he completed a 56 km marathon in 5 hours 34 minutes. There was no significant medical history. For the previous three years he had smoked an average of 10 cigarettes per day. At autopsy, the heart weighed 350 grams and there was evidence of subendocardial interstitial fibrosis in the left ventricle. The right ventricle showed adipose infiltration and scanty subendocardial myofibrillar degeneration. The left anterior

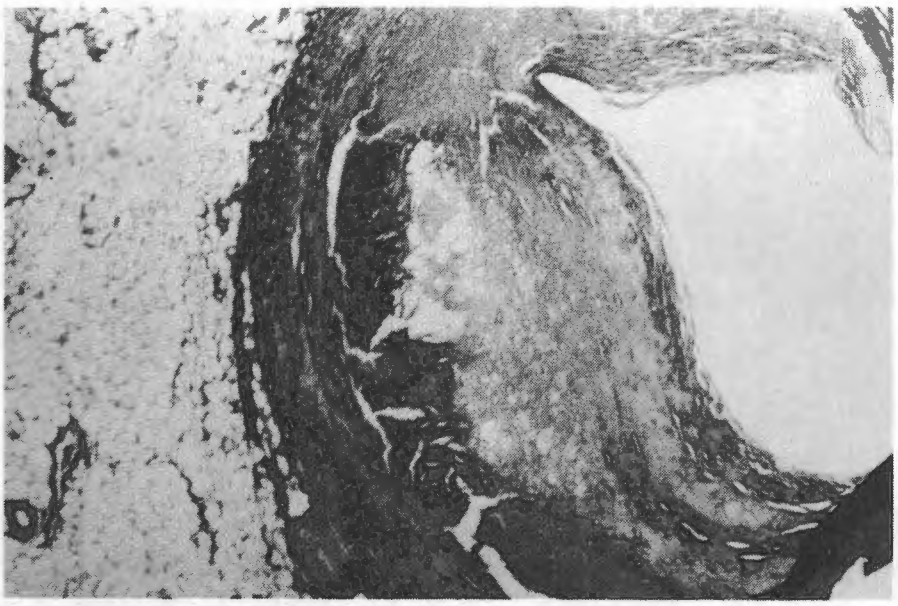

Figure 3.26

Case 8. Left anterior descending coronary artery shows more than 50% luminal narrowing by intimal atherosclerosis. (Phosphotungstic acid haematoxylin stain, x 30).

Figure 3.27

Case 8. Diagonal branch of the left anterior descending coronary artery shows severe luminal reduction by concentric intimal fibrosis. Scanty lipid is present. This marathon runner was asymptomatic, had been running for 7 years and was only 36 years old. (Haematoxylin and eosin, x 50).

descending branch of the left coronary artery showed 25 to 50% atherosclerotic narrowing. The circumflex branch, the mainstem of the left coronary artery and the right coronary artery had no appreciable narrowing.

CASE 10. (Case still under review)

A 29 year old runner,who was known to be hypertensive and who completed the 1980 Comrades Marathon, died suddenly whilst on a training run. For some months he had been troubled by exercise-induced chest pain, the nature of which had not been established.

At autopsy, the heart weighed 420 grams and there was a total occlusion of the anterior descending coronary artery about 2,5 cm from its origin (Fig. 3.28). The distal vessel and the circumflex coronary artery were free of atherosclerosis, but the right coronary artery showed areas of Grade 1-2 (25-50%) atherosclerotic narrowing.

The left ventricle showed concentric hypertrophy. Histology showed an area of healed myocardial infarction involving the inner 2/3rds of the antero-septal wall of the left ventricle. Additional sections taken from the left ventricular septum and free wall showed widespread areas of myofibre disarray sufficient to warrant a diagnosis of hypertrophic cardiomyopathy[407].

A case of hypertrophic cardiomyopathy associated with sudden death during marathon racing has previously been described by us[397]. Other authors have also reported that hypertrophic cardiomyopathy is an important cause of sudden death in young athletes[408].

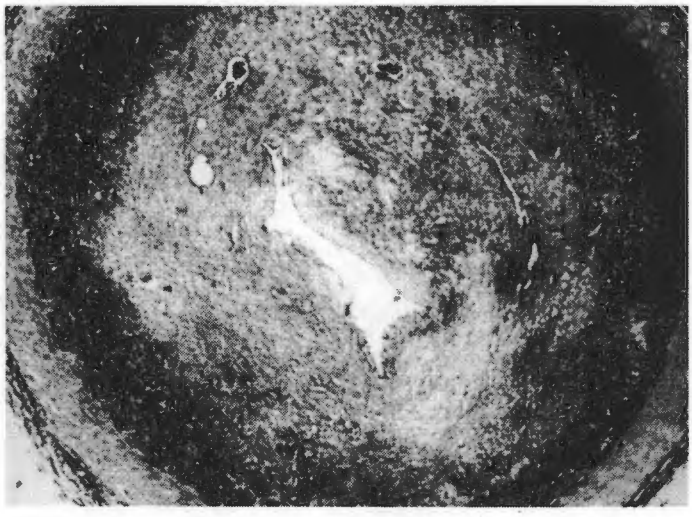

Figure 3·28

Case 10. Anterior descending coronary artery showing near-total occlusion approximately 2·5 cm from its origin.

B. Relevance of these cases to the "Bassler Hypothesis".

These cases confirm that autopsy-proved coronary atherosclerosis can occur in marathon runners, and that such disease may prove fatal. They thus disprove[41] the "Bassler hypothesis" as it was originally stated.

C. Cases in which autopsies were not performed.

CASE 11.

A 35 year old man had been running for at least 10 years before his death in February 1974. His athletic achievements included a best standard marathon time of 2 hours 33 minutes in April 1973 and a time of 3 hours 24 minutes for a 53 km race in April 1972. In May 1971 he finished the Comrades Marathon in 45th position out of 925 competitors in a time of 6 hours 51 minutes. During 1972 and 1973 he ran a total of 7 653 km.

His running logbook records that he went running on 11 occasions during the first 3 weeks of January 1974. On 6 of these runs reference is made to the presence of chest pain or pain between the shoulder blades, or both. This was severe enough to force him to stop running on a number of occasions. During one 7 km time trial, at which his wife was present, she recalls that he was forced to stop running 5 or 6 times. A friend advised him to see a doctor but he declined attributing his problems to "unfitness". In this period he ran 44 and 64 km training runs. During this longer run he had severe chest pain that frequently forced him to stop.

On the day of his death, he went to work as usual but telephoned home at 4 o'clock in the afternoon to say he was going surfing. However, he arrived home 50 minutes later saying that he was "too breathless" to

surf properly. He went inside the house and 20 minutes later asked his wife to take him to the doctor immediately as he had severe chest pain. His wife recalled that he looked pale but was not sweating and did not complain of nausea. During the car ride to the doctor he requested his wife to drive faster as the chest pain was getting worse and that it was now present in his left hand, which felt paralyzed.

He was seen by a general practitioner who gave him an injection and informed his wife that he had had a heart attack. The patient was driven to hospital immediately where he was admitted to the Intensive Care Unit. A diagnosis of acute myocardial infarction was made on the clinical features and the absence of signs of rupture of an aortic aneurysm or massive pulmonary embolus or other causes of acute severe chest pain.

The patient died at 6.40 p.m. and the diagnosis on the death certificate was coronary thrombosis. No autopsy was performed and an incorrect report in a letter[409] was discounted on further investigation[410].

An electrocardiogram was taken on admission to hospital (Fig.3.29) and later reported as showing changes of acute inferior subendocardial ischaemia. Scaff[411] has claimed that the patient died of heatstroke with which diagnosis the electrocardiogram was considered to be compatible. This interpretation has been repeatedly re-stated[412-414] but can however be readily discounted because the patient had not been running on the day of his death, and the symptoms were quite incompatible with heatstroke[415,416].

In view of the strong likelihood that this patient came from a family with a genetic predisposition for coronary artery disease, members of his family were subsequently investigated. Of his 4 brothers, 2 have subsequently died suddenly within a period of 10 days of each other. In addition, it was found that his daughter has a type 2B hyperlipidaemia (cholesterol 375 mg/100 ml and triglycerides 271 mg/100 ml). Thus, it seems

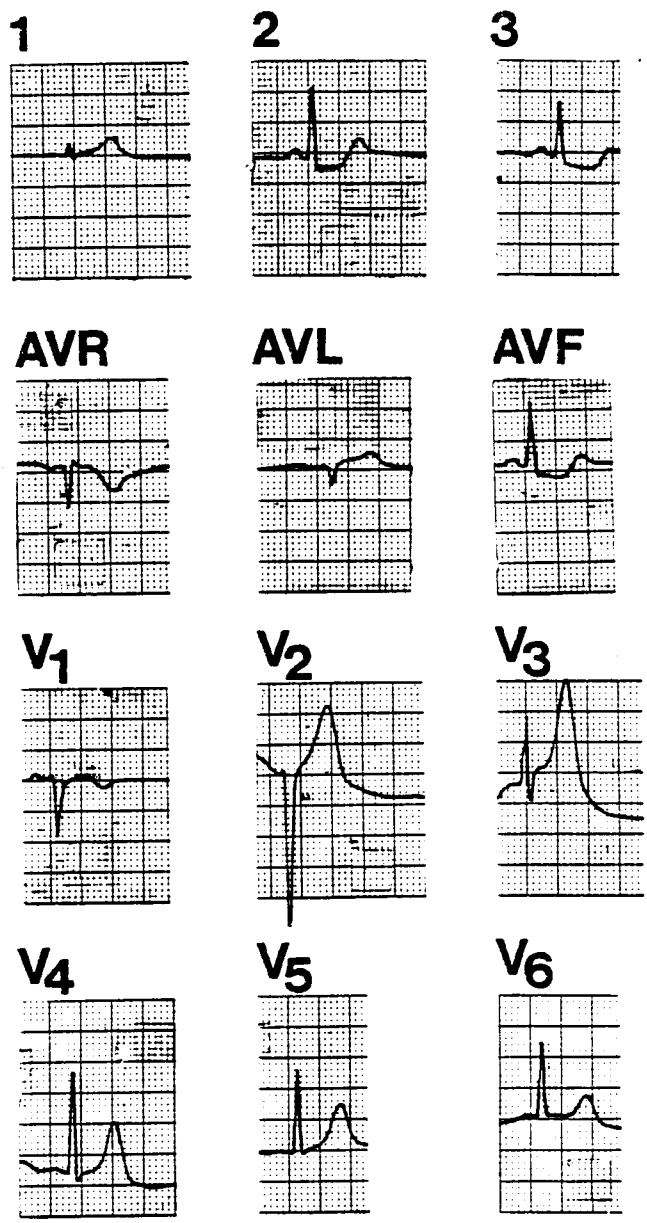

Figure 3. 29

Case 11. Resting electrocardiogram taken shortly before death showing ST segment depression in leads 2, 3 and AVF.

that the deceased does indeed come from a family with a genetic hyperlipidaemia and that marathon running could not protect against the fatal consequences of that disease.

CASE 12.

In October 1970, 16 months after completing the 1969 Comrades Marathon in 10 hours 33 minutes, a 47 year old athlete collapsed and died 80 metres short of the finish line of the 13 km Harrismith Mountain Race. He was immediately attended to by a physician who applied cardiopulmonary resuscitation, without success. The patient was declared dead on arrival at the nearest hospital.

There was no family history of heart disease and the patient had never complained of cardiac symptoms. Six months prior to his death, during a routine medical examination, a grade 3-4/6 systolic murmur was heard in the aortic area. The blood pressure was normal (140/90 mmHg). The chest X-ray was reported as showing left ventricular hypertrophy but the electrocardiogram was within normal limits. Serum cholesterol 252 mg/100ml (normal 135-250), triglycerides 136 mg/100 ml (normal 60-150) and uric acid 6,5 mg/100 ml (normal 2-6,5), were all normal. The cause of the aortic murmur was never clinically established, and no autopsy was performed.

CASE 13.

A 58 year old man, who had already completed 6 Comrades Marathons, felt unwell during the 1978 Comrades Marathon and visited a physician shortly thereafter. The electrocardiogram, which was subsequently misplaced, was reported to be compatible with a non-transmural myocardial infarction. The patient declined an offer to come to Cape Town for cardiological evaluation, and continued to train for and to run marathons.

In February 1979 he was found dead in his bed, 36 hours after completing a 42 km marathon. No autopsy was performed.

CASE 14.

A 53 year old male started running in 1975, and completed a number of standard and ultra-marathon races, including 2 Comrades Marathons.

In May 1980, a few hours after completing an uneventful 42 km training run as part of a 160 km training week, he died suddenly whilst resting on his bed. No autopsy was performed.

Other runners, who had seen this athlete running immediately prior to his death, reported that he looked well. He had not complained of any abnormalities either to these runners or to his wife after he had returned home.

3.4 SUMMARY AND CONCLUSIONS

These studies show that coronary atherosclerosis and sudden cardiac death do occur in trained marathon runners. This finding has been confirmed by 2 more recent studies[417,418]. They also show that such deaths occur not infrequently (a minimum of 5 cases in the past 2 years in South Africa) and that, at least in this country, unless special interest is shown in the case, most marathon deaths will be inadequately autopsied. It should be noted that, despite "worldwide surveillance", in ten years, Dr. Bassler[419] was able to collect only 4 such autopsy cases in North American marathoners. This suggests that prior to the more recent interest[417,418], a similar problem regarding adequate autopsies in marathon runners also existed in the United States. This would be one explanation why the "Bassler hypothesis" went unchallenged for as long as it did.

It must be emphasized that although these studies disprove the extreme hypothesis that exercise training can provide absolute protection against coronary atherosclerosis or sudden cardiac death, they provide no information for a more general thesis that marathon running or other prolonged exercise might provide partial protection against this disease. Such a possibility deserves ongoing epidemiological attention.

A number of additional features, not directly relevant to the specific topic investigated by this thesis, became apparent during these studies. They are reviewed here as they relate to the general topic of exercise and the heart.

The presence of warning symptoms prior to myocardial infarction or sudden death.

The majority of athletes had continued training, or indeed racing, despite warning symptoms. Two athletes (Cases 1 and 3) completed marathon races with symptoms, one of these athletes (Case 1) running more than 30 km after the onset of exertional discomfort to complete the 90 km Comrades Marathon. When told that he had suffered a heart attack, Case 4 refused to be hospitalized and went home and mowed his lawn for 5 hours with severe chest pain. Case 5 "ran through" chest pain and dyspnoea to complete the Comrades Marathon. Five months later despite severe chest pain, he continued to run a 16 km race and collapsed at the finish. Case 6 persisted in walking/jogging even when he had unstable angina. One month before his subsequent death during a 24 km road race, Case 7 visited his physician complaining of a lack of energy. Case 10 continued to run with chest pain after a medical practitioner had failed to establish the cause of this pain. Case 11, who died shortly after the onset of severe chest pain, had continued training for 3 weeks, including a 64 km run with chest pain which he ascribed to "unfitness". Case 13 continued to train and run marathons with chest pain, and declined to undergo medical evaluation. Another marathon runner with hypertrophic cardiomyopathy (not reported here), who died during a marathon race, had suffered previous attacks of chest pain and had remarked that he felt unwell and wished to quit during the fatal race[397,416].

Denial of premonitory symptoms has since been noted by other authors. Thompson, Stern, Williams et al[417] reported that 6 of 13 runners with coronary heart disease, who died suddenly during or shortly after exercise, had premonitory symptoms. In none was there chest pain, but 3 runners had been troubled by abdominal discomfort. Two of our runners who developed myocardial infarction during marathon racing (Cases 1 and 3) also presented with abdominal symptoms. Thompson and his colleagues point out that failure to appreciate gastric pain as being of possible cardiac origin may postpone diagnostic evaluation, as occurred in both our cases.

Colt[420] has also described 2 marathon runners who continued to train and race despite symptoms, one athlete continued to run for 6 km with chest pain before collapsing in the 1978 New York City Marathon with extensive anteroseptal myocardial infarction. Another runner, who had previously lost consciousness after a training run, died one month before the same New York marathon. One week before his death, this runner had put the following words on his T shirt: "You haven't really run a good marathon until you drop dead at the finish line - Pheidippides". An American congressman, who had completed the Boston Marathon 7 times and who died after a 19 km training run, had been advised by a cardiologist to stop running, pending further cardiovascular evaluation for angina pectoris and an abnormal exercise electrocardiogram. The congressman who was aware of the "Bassler hypothesis" apparently believed that his accomplishments as a marathon runner immunized him against heart disease.

In a study of sudden death in 29 young athletes[408], 19 of whom had either hypertrophic cardiomyopathy or idiopathic concentric left ventricular hypertrophy, and only 3 of whom had coronary artery disease, premonitory symptoms were uncommon, being present in only 8 cases. Thus, although

our study suggests that premonitory symptoms are likely to be present in older athletes at risk of sudden death, most of whom will have coronary atherosclerosis, the study of Maron and his colleagues suggests that, in younger athletes, premonitory symptoms may be less likely, possibly because hypertrophic cardiomyopathy, the more common fatal cardiac disease in that age group, may not cause obvious premonitory symptoms.

The role of overtraining and marathon racing.

Another feature that needs to be considered is whether overtraining or alternatively marathon racing had contributed to the subsequent sudden deaths or heart attacks of these marathoners. Cases 1, 3 and 6 had all been undergoing periods of particularly intensive training before their heart attacks. In the week leading up to his death, Case 14 had run 160 km, a training "mileage" quite incompatible with his modest athletic ability. It should be noted that Case 4 developed severe angina pectoris one week after completing a standard marathon. Cases 5 and 10 developed significant angina pectoris in the period immediately following the Comrades Marathon, Case 6 developed unstable angina one month after completing a standard marathon, and Case 13 died within 36 hours of completing a marathon.

Further studies are indicated to determine whether these events were purely coincidental or whether marathon racing might actually be detrimental for some persons with advanced coronary artery disease.

High levels of physical fitness do not guarantee the absence of significant cardiovascular disease.

Despite subsequently documented severe coronary artery disease, many of these athletes were able to successfully complete marathon or longer races. Cases 4, 5 and 7 all completed the Comrades Marathon within months of being shown to have advanced triple vessel coronary artery disease. At autopsy, one month after he had completed a marathon, the heart of Case 6 showed complete occlusions of all 3 major epicardial coronary arteries.

A disturbing feature was the rapidity with which severe cardiac symptoms developed in some athletes. Thus, cases 4, 5 and 6 all developed severe, incapacitating angina pectoris of rapid onset.

Practical implications

These studies have 4 practical implications for both runners and physicians.

First, runners should not assume that attaining the marathon distance will ensure total immunity from coronary heart disease nor that attaining that distance after myocardial infarction will necessarily prevent progression of the disease.

Second, runners should seek medical advice and not force themselves when exertional symptoms develop, as occurred in the majority of our cases.

Third, when consulted by symptomatic marathon runners, physicians should not exclude the possibility of coronary heart disease simply because the athlete is "physically-fit". Cases 6 and 10 provide good examples of this. Shortly before their deaths, both consulted medical

practitioners with vague (Case 6) and specific (Case 10) cardiac symptoms, but in neither was the correct diagnosis established prior to death. It would seem that symptoms sufficiently severe to interfere with the athlete's running performance definitely warrant a complete cardiac evaluation, including a maximum exercise stress test, echocardiography and even possibly ventriculography with provocative testing to define the non-obstructive form, if hypertrophic cardiomyopathy is considered a diagnostic possibility.

Fourth, in order that the preventable aspects of sudden death in athletes be better understood, it is essential that, in all such cases, more thorough autopsies should be performed.

CHAPTER 4.

EXERCISE TRAINING AND MYOCARDIAL RESISTANCE TO VENTRICULAR FIBRILLATION.

STUDIES IN THE ISOLATED PERFUSED RAT HEART MODEL.

4.1 INTRODUCTION

The studies reported in the previous chapter have established that even the high levels of regular exercise maintained by marathon runners cannot absolutely protect them against either coronary atherosclerosis or sudden cardiac death. However, the likelihood that any such extreme medical hypothesis will ultimately turn out to be true, must always be extremely remote. Thus the "unequivocal destruction" of the Bassler hypothesis[41] should not be allowed to detract from the more realistic possibility that exercise training might indeed provide *partial* protection against either coronary atherosclerosis or sudden cardiac death, or both. But the further study of these specific questions will require additional epidemiological studies that must circumvent the problems detailed in section 2.2A - studies to which, at present, I have no access.

Therefore, to further my own studies of this topic, I chose an animal model to investigate the effects of exercise training on myocardial electrical stability during experimental interventions that are believed to mimic conditions present during heart attack. Currently, there are 2 such animal models:

(i) The *in vivo* dog model[422] in which bipolar stimulating electrodes are introduced into the left ventricular myocardium during thoracotomy. When the animal has fully recovered from the operation, its ventricular fibrillation threshold (VFT) may be measured by stimulating the ventricular electrodes at a current, measured in milliamperes, sufficient to produce ventricular fibrillation.

(ii) The isolated perfused rat heart model[423].

I chose the latter *in vitro* model to compare the ventricular fibrillation thresholds of isolated hearts from running-trained rats with

those of hearts from control animals during control perfusions, and under conditions of hypoxia and acute regional myocardial ischaemia, to determine whether exercise training could increase myocardial electrical stability under these experimental conditions.

4.2 MATERIALS AND METHODS.

Three weeks after weaning, young male Wistar-Weissman rats were randomly assigned to either an exercising or a control group and were housed and fed under identical conditions. Rats assigned to the exercising group completed the training programme described in Appendix 1.A.

Beginning after 9 weeks' training, rats from either the control or trained group were anaesthetized with ether in a vacuum bowl and when asleep, 10 µl of heparin (Pularin$^{(R)}$, Glaxo-Allenburys (SA) Pty Ltd., Wadeville, 1000 u/ml) was injected into the femoral vein. The thoracic cavity was opened, the heart exposed and gently lifted between thumb and forefinger. Parts of the lungs and the great arteries were then cut, the heart rapidly excised and immediately placed in ice-cold ($4^{o}C$) Krebs-Henseleit buffer. As soon as the heart had stopped beating, it was removed from the buffer, the aorta was incised immediately proximal to the aortic arch, and the opened aorta was slipped onto the aortic cannula of the isolated heart perfusion system (for more details see next chapter). Extreme care was taken to insure that there were no bubbles in the aorta when it was mounted on the cannula. Hearts from trained and control rats were perfused alternatively so that an equal number of hearts from each group were studied on each experimental day. The perfusate used was a modified Krebs-Henseleit buffer solution (Appendix 1.B) with a $CaCl_2$ concentration of 1,1 mM, aerated with either 95% O_2:5% CO_2 or with 95% room air and 5% CO_2 during studies of hypoxia. The perfusate substrate was 11,1 mM D(+) glucose (Merck, Darmstadt, West Germany) plus insulin (NUSO Neutral Insulin$^{(R)}$, Wellcome Foundation) at a concentration of 2 u/litre. The perfusion pressure was 100 cm H_2O. In those experiments in which myocardial glycolytic rates were measured ^{3}H-glucose (100 µl/litre

perfusate) was added to the perfusion fluid, and the coronary effluent analyzed for its $^{3}H_2O$ content according to the techniques described in Appendix 1.C.

A. Ventricular fibrillation threshold measurements during control perfusions.

The technique for measuring the ventricular fibrillation threshold in the isolated perfused rat heart has been described in detail by workers in this laboratory[423]. Following mounting of the heart on the aortic cannula of the isolated perfusion system, thin platinum electrodes were inserted into the apex and base of the left ventricle with the anode at the apex, for the delivery of square wave stimuli of 2 msec duration (Grass S88 physiologic stimulus generator). For hearts used in the studies of regional ischaemia, a 5-0 silk suture with an atraumatic needle was passed deep to the left main coronary artery within 2 mm of where the artery emerges adjacent to the left atrium, according to the technique described by Kannengiesser,Lubbe and Opie[424]. The ventricular fibrillation threshold was measured by applying a single train of 10 stimuli of 200 msec duration across the T wave starting 10 msec after the onset of the R wave. The heart was stimulated every 30 seconds unless ventricular fibrillation occurred, in which case the next stimuli was applied only after 60 seconds. The current strength was increased by increments of 2,5 milliamperes (mA) until ventricular fibrillation,consisting of 6 or more repetitive ectopic cycles with irregular form, developed. The ventricular fibrillation threshold was defined as the lowest current which produced ventricular fibrillation on 3 occasions, and which did not produce fibrillation at a current of 0,5 mA lower.

The hearts were allowed a period of 15 minutes for stabilization before measurement of heart rate, from the oscilloscope, and coronary flow rate, by collection in a graduated measuring cylinder a timed sample of coronary effluent from the heated chamber below the heart. The ventricular fibrillation threshold was then measured, and this was taken to represent the value for the control perfusion.

B. Ventricular fibrillation threshold measurements during acute regional ischaemia.

After the ventricular fibrillation threshold had been measured under control conditions, hearts from 12 trained and 14 control rats were exposed to acute regional ischaemia by abrupt tightening of the ligature surrounding the anterior descending coronary artery. Beginning 5 minutes after ligation, ventricular fibrillation thresholds were measured every 30 seconds for a further 10 minutes in hearts from 6 trained and 7 control rats, and for 15 minutes in another 6 trained and 7 control rat hearts.

In the group perfused for a total of 20 minutes regional ischaemia, the experiments were terminated by removal of hearts from the aortic cannula following injection of disulphan blue dye into the perfusate through the aortic cannula. This technique differentiates the ischaemic from the non-ischaemic zone of the left ventricle[425]. The ischaemic zone was then dissected out, weighed, and recorded as a percentage of the total left ventricular wet weight.

In the group of hearts perfused for a total of 15 minutes regional ischaemia, the experiments were terminated by freeze-clamping the hearts in Wollenberger tongs[426] precooled in liquid nitrogen. Hearts were then individually stored in liquid nitrogen awaiting biochemical analysis by methods described in Appendix 1.D.

C. Ventricular fibrillation threshold measurements during hypoxia and during hypoxia with catecholamine stimulation.

After measurement of the control ventricular fibrillation thresholds, a further series of hearts from 8 trained and 8 control rats were exposed to 15 minutes hypoxic perfusions during which the ventricular fibrillation thresholds were measured every 30 seconds.

After the initial 15 minutes'hypoxic perfusions, 1×10^{-8}M isoproterenol (Isuprel$^{(R)}$ - Winthrop Laboratories) was infused for a further 15 minutes during which hypoxia was maintained. Ventricular fibrillation thresholds were again measured during this period, and the experiments were terminated by freeze-clamping the hearts in pre-cooled Wollenberger tongs.

In these experiments, coronary effluent was samples every 5 minutes and its $^{3}H_2O$ content measured for calculation of glycolytic rates as described in Appendix 1.C.

D. Biochemical measurements.

The tissue contents of adenosine triphosphate (ATP), phosphocreatine (PCr), glycogen, lactate and 3',5' cyclic adenosine monophosphate (cyclic AMP) were measured in freeze-clamped hearts homogenized under liquid nitrogen by methods described in Appendix 1.D. Glycolytic rates were calculated according to the formulae described in Appendix 1.F.

4.3 EXPERIMENTAL RESULTS.

A. Ventricular fibrillation thresholds during acute regional ischaemia.

Fig. 4.1 shows the ventricular fibrillation thresholds in trained and control hearts before and after coronary artery ligation. It will be seen that at all times after coronary artery ligation, the ventricular fibrillation thresholds are significantly higher in hearts from trained rats ($p < 0,05$).

Table 4.1 lists additional observations made in these experiments. There were no differences in heart rates or coronary flow rates either before or after coronary artery ligation, and the infarct sizes expressed as a percentage of total left ventricular wet weights were similar in both groups. However, ventricular fibrillation thresholds fell significantly sooner after coronary artery ligation in control, than in trained hearts.

Table 4.2 lists the tissue levels of ATP, PCr, glycogen, lactate and cyclic AMP in trained and control hearts clamped after 15 minutes regional ischaemia. The only significant difference was that tissue cyclic AMP levels were significantly lower in the ischaemic left ventricular zone of hearts from trained animals ($p < 0,02$).

B. Ventricular fibrillation thresholds during hypoxia, and during hypoxia with catecholamine stimulation.

In this series of experiments, the ventricular fibrillation thresholds were significantly higher in hearts from trained animals during

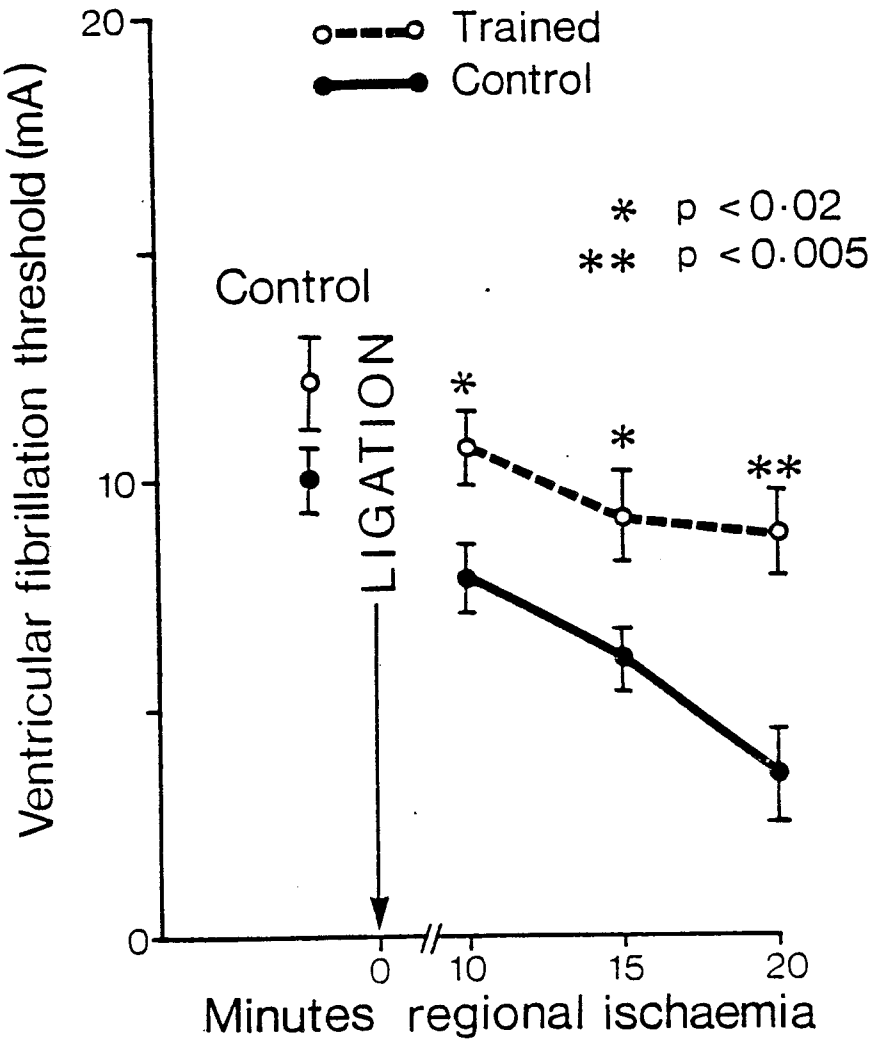

Figure 4.1

Effects of coronary artery ligation on ventricular fibrillation thresholds of hearts from trained and control rats.
Note that at all times after coronary artery ligation, ventricular fibrillation thresholds are higher in trained hearts.

TABLE 4.1

HEART RATES, CORONARY FLOW RATES, TIMES TO FALL IN VENTRICULAR FIBRILLATION THRESHOLDS (VFT) AND INFARCT SIZES OF HEARTS FROM TRAINED AND CONTROL RATS EXPOSED TO ACUTE REGIONAL ISCHAEMIA.

	TIME TO FALL IN VFT (min)	CONTROL HEART RATES (beats/min)	HEART RATES AFTER 20 MIN REGIONAL ISCHAEMIA (beats/min)	CONTROL CORONARY FLOW RATES (ml/min)	CORONARY FLOW RATES AFTER 20 MIN REGIONAL ISCHAEMIA (ml/min)	INFARCT SIZES (%)
Trained rats	11,5 ±1,0 (12)	185,3 ±5,6 (12)	172,5 ±8,4 (12)	7,9 ±0,4 (12)	5,3 ±0,4 (12)	36,9 ±1,8 (12)
Control rats	8,4 ±0,8 (14)	180,6 ±6,1 (14)	172,4 ±6,1 (14)	8,4 ±0,6 (14)	5,1 ±0,5 (14)	39,3 ±2,3 (14)
P value	<0,05	NS	NS	NS	NS	NS

Note that the heart rates, coronary flow rates and infarct sizes are not different between trained and control hearts, but that the mean time to the initial fall in VFT is significantly longer in trained hearts.

Values are Mean ± SEM (number of measurements).
Weights of rats used in these studies ranged between 230 and 330 gms.
Fresh weights of perfused hearts were in the range 0.5 to 0.6 gms.

TABLE 4.2

TISSUE ATP, PCr, GLYCOGEN, LACTATE AND cAMP LEVELS IN NORMAL AND ISCHAEMIC ZONES OF HEARTS FROM TRAINED AND CONTROL RATS AFTER 15 MINUTES REGIONAL ISCHAEMIA.

	ATP µmol/g	PCr µmol/g	GLYCOGEN µmol glucose equiv/g	LACTATE µmol/g	cAMP nmol/g
NORMAL TISSUE					
Trained rats	4,0 ±0,1 (6)	5,8 ±0,4 (6)	21,0 ±1,1 (6)	1,4 ±0,5 (6)	0,36 ±0,1 (6)
Control rats	4,3 ±0,5 (6)	6,1 ±0,8 (6)	20,2 ±1,2 (6)	2,1 ±0,5 (6)	0,41 ±0,1 (6)
P value	NS	NS	NS	NS	NS
ISCHAEMIC TISSUE					
Trained rats	2,9 ±0,2 (6)	2,5 ±0,5 (6)	18,1 ±1,5 (6)	15,1 ±2,0 (6)	0,38 ±0,1 (6)
Control rats	3,5 ±0,3 (6)	2,7 ±0,6 (6)	17,4 ±1,7 (6)	16,6 ±3,2 (6)	0,48 ±0,1 (6)
P value	NS	NS	NS	NS	<0,02

Note that cyclic AMP levels are significantly lower in the ischaemic left ventricular zone of trained hearts.

Values are Mean ± SEM (number of measurements).

the control perfusions, during hypoxia, and during hypoxia combined with isoproterenol infusion (fig. 4.2).

Table 4.3 lists the additional observations made during these experiments. It shows that there were no significant differences between trained and control animals, in heart rates or coronary flow rates during either the control perfusions, or during hypoxia or during hypoxia combined with isoproterenol infusion. There were also no significant differences in any metabolic measurements between hearts from trained and control rats (Table 4.4). Glycolytic rates of both trained and control hearts increased progressively during hypoxic perfusions, and were higher, but not significantly, in hearts of trained rats (Fig. 4.3).

One explanation for the significantly higher ventricular fibrillation thresholds in trained hearts during control perfusions in this, but not in the first experimental series (Fig. 4.1) is a training duration effect. Thus, the rats used in this series of experiments had been trained for between 12 and 16 weeks whereas, in the first study, the rats had trained only for between 9 and 12 weeks. When all the ventricular fibrillation thresholds during control perfusions are combined, the value for the trained hearts is 13,72 $\pm$ 1,09 mA (20 experiments) and for the control hearts 9,57 $\pm$ 0,57 mA (22 experiments). This difference is statistically significant ($p < 0,005$).

4.4 DISCUSSION AND CONCLUSIONS

These studies are the first to show that ventricular fibrillation thresholds are increased in hearts from trained animals during control perfusions, during hypoxia and during acute regional ischaemia. The only previous report that has relevance to this study is that of Ammann, Meesmann, Schley et al[427], who reported that in response to acute circumflex

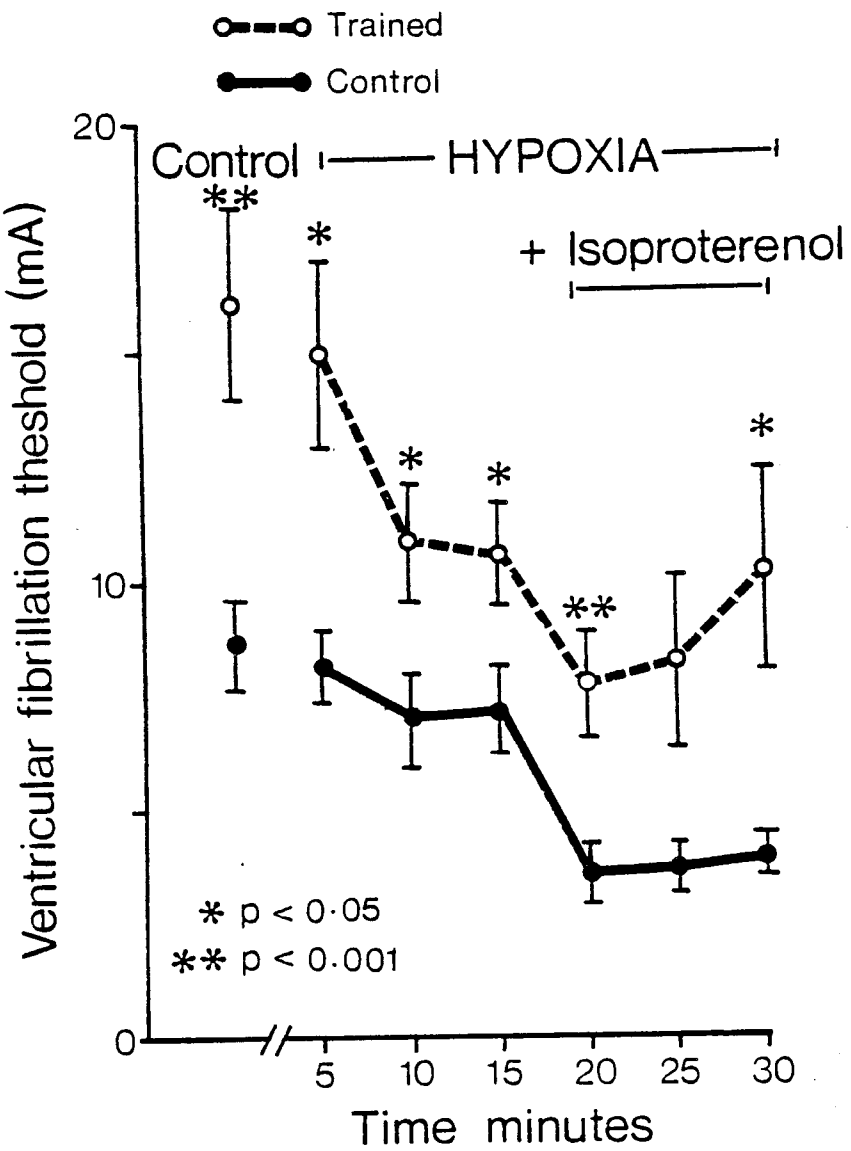

Figure 4.2

Effects of hypoxia, and of hypoxia combined with isoproterenol infusion on ventricular fibrillation thresholds of trained and control hearts.
Note that at all times, ventricular fibrillation thresholds are higher in trained hearts.

TABLE 4.3

HEART RATES AND CORONARY FLOW RATES OF HEARTS FROM TRAINED AND CONTROL RATS DURING CONTROL PERFUSIONS AND DURING HYPOXIA.

	CONTROL HEART RATES (beats/min)	HEART RATES DURING HYPOXIA (beats/min)	HEART RATES DURING HYPOXIA PLUS ISOPROTERENOL INFUSION (beats/min)	CONTROL CORONARY FLOW RATES (ml/min)	CORONARY FLOW RATES DURING HYPOXIA (ml/min)	CORONARY FLOW RATES DURING HYPOXIA PLUS ISOPROTERENOL INFUSION (ml/min)
Trained rats	187,4 ±6,9 (8)	198,3 ±12,4 (7)	274,8 ±17,7 (8)	7,5 ±0,5 (8)	15,7 ±0,8 (8)	21,7 ±0,8 (8)
Control rats	198,8 ±8,5 (8)	184,8 ±11,3 (8)	252,4 ±11,3 (7)	8,4 ±0,5 (8)	16,0 ±1,7 (8)	21,1 ±1,2 (7)
P value	NS	NS	NS	NS	NS	NS

Values are Mean ± SEM (number of measurements).

TABLE 4.4

TISSUE ATP, PCr, GLYCOGEN AND cAMP LEVELS IN HEARTS FROM TRAINED AND CONTROL RATS AFTER HYPOXIC PERFUSIONS.

	ATP µmol/g	PCr µmol/g	GLYCOGEN µmol glucose equiv/g	cAMP nmol/g
Trained rats	3,1 ±0,2 (8)	4,7 ±0,3 (8)	16,0 ±0,6 (8)	0,35 ±0,1 (8)
Control rats	3,3 ±0,1 (7)	4,7 ±1,4 (7)	14,3 ±0,7 (7)	0,41 ±0,1 (7)
P value	NS	NS	NS	NS

Values are Mean ± SEM (number of measurements)

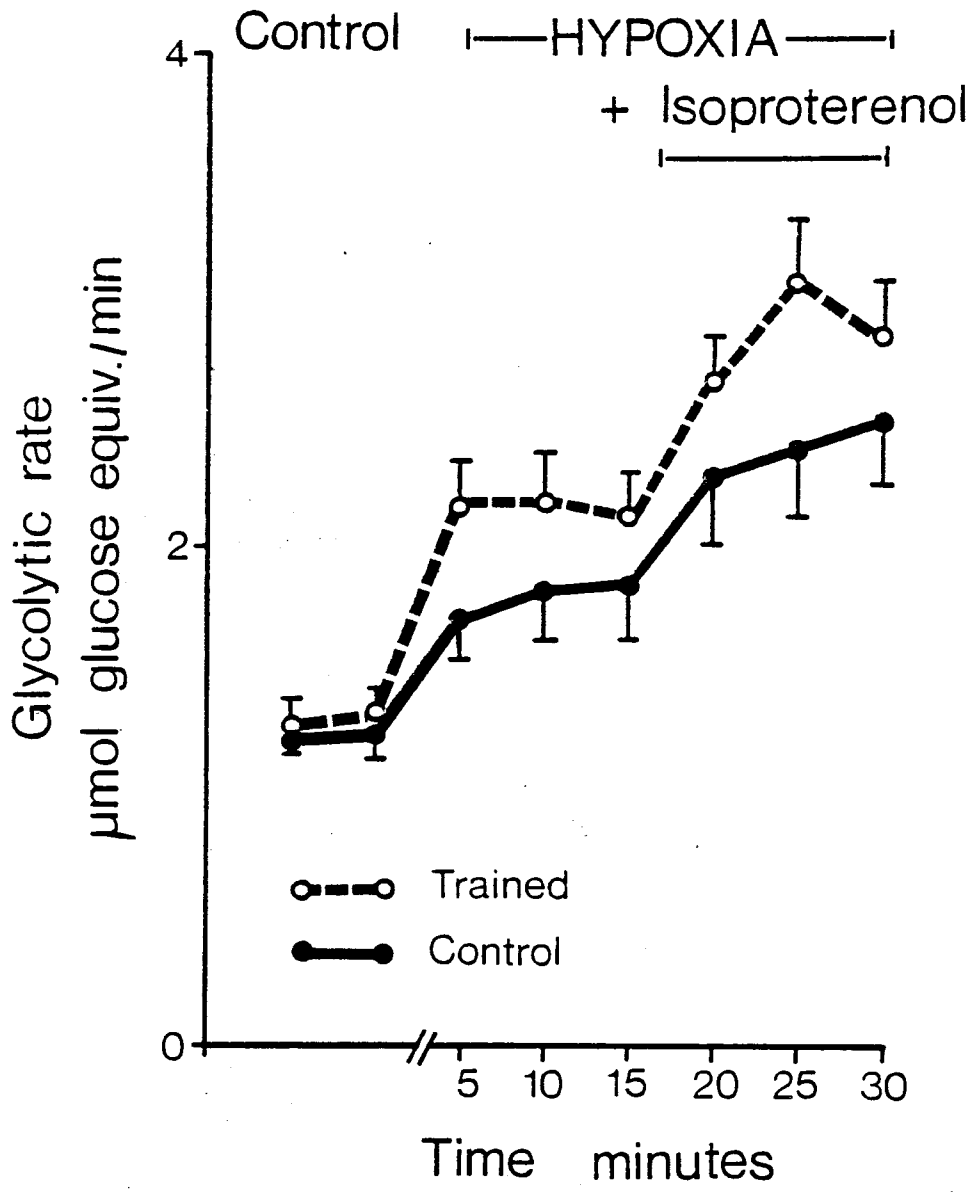

Figure 4.3

Effects of hypoxia, and of hypoxia combined with isoproterenol infusion on glycolytic rates of trained and control hearts.
Note that glycolytic rates are not different between trained and control hearts, and that glycolytic rates are increasing as ventricular fibrillation threshold are falling (Fig 4.2).

coronary artery ligation, 8 of 8 untrained dogs developed ventricular fibrillation, whereas only 8 of 16 trained dogs developed this fatal arrhythmia.

In this study, the increased thresholds in trained hearts during acute regional ischaemia could not be explained on the basis of differences in heart rates, in coronary flow rates, or in tissue levels of high energy phosphates, glycogen or lactate. There was also no evidence that, in this model, exercise training had caused reduced infarct sizes in response to coronary artery ligation as found by McElroy, Gissen and Fishbein[136]. Cyclic AMP accumulation in the ischaemic left ventricular zone was however significantly less in trained hearts, a finding which is in agreement with the work of Kleitke, Wollenberger, Krause et al[226] who reported that, in response to acute global ischaemia, hearts from swimming-trained rats had significantly lower cyclic AMP levels than did control rats. In view of the hypothesis linking myocardial cyclic AMP levels and reduced ventricular fibrillation thresholds in this model[423], the lower cyclic AMP levels in the ischaemic left ventricular zones of hearts from trained rats could explain their higher ventricular fibrillation thresholds during regional ischaemia. However, the magnitude of the cyclic AMP difference is quite small.

The higher ventricular fibrillation thresholds in trained hearts during hypoxia and during hypoxia with catecholamine stimulation could also not be explained on the basis of differences in heart rates, tissue metabolite levels or glycolytic rates. There was also no evidence that, in this model, training increases coronary flow responses to hypoxia, as found by Spear, Koerner and Terjung[332].

The finding that, in this model, exercise training increases myocardial resistance to ventricular fibrillation, is not necessarily directly applicable to the human situation, in which ventricular fibrillation occurs

spontaneously and not in response to an exogenous electrical stimulus as used in these studies. Indeed, the evidence that exercise training alters myocardial electrical stability in humans, is conflicting.

Thus, Blackburn, Taylor, Hamrell et al[428] provide suggestive evidence that after an 18-month exercise training programme in humans, the exercise threshold for premature ventricular contractions increased, whereas the proportion of men with ventricular ectopic activity, and the frequency of premature ventricular complexes per man, both decreased. Similarly Viitasalo, Kala, Eisalo and Halonen[429] have reported that healthy, physically-active men had lower rates of premature ventricular contractions during both exercise testing and jogging, than did either sedentary healthy men or men with previous myocardial infarctions. The authors discussed the potential selective factors and the different psychological stresses that could have been present during exercise in the different groups, and concluded that their study was not absolute proof that exercise training reduced ventricular ectopy. In a longitudinal study that avoided these problems, Ekblom, Hartley and Day[430] found that regular physical activity was not a factor reducing premature ventricular contractions either at rest or during exercise.

In summary, the studies reported in this chapter are compatible with the epidemiological evidence which suggests that exercise training increases resistance to sudden coronary death. Furthermore, they give added impetus for research to clarify the paradox described by Viitasalo, Kala, Eisalo and Halonen[429]: "We are in a most unhappy situation, where ventricular premature beats of similar outlook might be both an indication and a contraindication for prescribing physical exercise, because differentiation between non-life threatening and life-threatening ventricular premature beats has not been satisfactorily clarified". Thus, there is a need

for additional human research that will identify which group or premature ventricular contractions are life-threatening, and whether such ventricular ectopy can be reduced by appropriate exercise training. In the animal model, further research should attempt to identify the biochemical basis of this training-induced increase in ventricular fibrillation threshold and, in particular, should evaluate the role of the altered myocardial calcium transport that results from exercise training (Chapter 6).

CHAPTER 5.

MYOCARDIAL ADAPTATIONS TO EXERCISE TRAINING - PART 1.

METHODOLOGY AND RESULTS OF PRELIMINARY STUDIES IN THE ISOLATED PERFUSED WORKING RAT HEART MODEL.

5.1 INTRODUCTION

The studies described in the previous chapter show that the rat heart undergoes training-induced adaptations that increase its resistance to electrically-induced ventricular fibrillation. The exact biochemical explanation for these changes was not established. Given the current uncertainty surrounding the biochemical basis of ventricular fibrillation[431], I did not feel that a direct continuation of this work would necessarily provide additional relevant information. I therefore chose to continue this research by studying the myocardial functional and metabolic adaptations to exercise training.

In the conclusion to Chapter 2, it was shown that the only studies which have consistently shown that exercise training improves the mechanical performance of the heart are those which have been performed with the heart working at, or near, its maximum. When hearts have been studied under resting conditions, adaptations have not usually been apparent. Thus, the model used to study the cardiac adaptations to exercise training must allow measurements to be made when the heart is under maximum load.

The isolated perfused working rat heart model is one experimental model that fulfills this criterion. The particular advantages of this model are that cardiac function and metabolism can be studied

simultaneously under tightly controlled experimental conditions, in which the heart functions free of neurohumoral control and is perfused through its own vascular bed. However, as yet, no previous study has systematically determined what perfusion conditions are necessary to elicit maximum mechanical performance in this model. In particular, little attention has been paid to the effects that different perfusion substrates have on the function of the maximally-working perfused rat heart.

In the studies reported in this chapter, I therefore set out to determine what perfusion conditions are necessary to insure that the isolated perfused rat heart is working at its maximum. Thereafter, I performed additional studies to explain what factors limit maximum mechanical performance in this model. This additional information was incorporated into the model for the studies of the myocardial adaptations to exercise training reported in Chapter 6.

5.2 THE ISOLATED PERFUSED WORKING RAT HEART MODEL.

A. Historical development of the model.

Langendorff[432] in 1895 described the first model in which rat hearts were perfused in an isolated system. In the perfusion technique that now bears his name, the heart is removed from the donor rat and mounted on a single aortic cannula. The coronary arteries are then perfused retrogradely from a reservoir, the fluid level of which is usually maintained at between 65 and 100 cm above the aortic valve. The pressure exerted by this fluid keeps the aortic valve closed during left ventricular contraction. Thus, the heart contracts isovolumically and does not eject fluid, the so-called non-working, Langendorff preparation.

In 1967 Neely, Liebermeister, Battersby and Morgan[433] advanced this technique to include cannulation of the left atrium. After the aorta had been mounted by the Langendorff technique, the left atrium was attached to a second cannula. Fluid that enters the left atrium via this cannula crosses the mitral valve into the left ventricle, which spontaneously ejects the fluid into the aortic column, from the top of which the fluid drips into an oxygenated reservoir. Re-oxygenated fluid is then re-circulated by a peristaltic pump to the left atrium. Therefore, in contrast to the Langendorff preparation, in this model the left ventricle ejects a cardiac output, the so-called working heart preparation.

B. Factors controlling heart function in the model - left atrial filling pressure, heart rate, aortic column height and perfusion substrates.

The amount of work performed by the isolated rat heart is principally determined by 3 factors - the left atrial filling pressure, the resistance produced by the aortic column, and the heart rate. No previous investigator has clearly defined that combination of these 3 factors which, in this model, elicits maximum heart function.

In Neely, Liebermeister, Battersby and Morgan's original paper[433], the highest workloads were achieved with atrial filling pressures of 20 cmH_2O. In later studies[434], heart work at this atrial filling pressure was further increased by reducing the calibre of the aortic column,thereby increasing the resistance to aortic flow. In this way peak left ventricular pressures of 160 mmHg were achieved.

In their studies using this model to quantify the effects of exercise training on myocardial performance, Scheuer and his colleagues (Section 2.3D) have routinely used a maximum atrial filling pressure of 20 cmH_2O, an aortic column height of 85 cm and heart rates of 330 beats/min. Under these conditions, peak left ventricular pressures are lower than those reported by Neely and his colleagues[434]. More recently, Miller[435] has perfused hearts from diabetic and control rats at left atrial filling pressures up to 30 cmH_2O with an aortic column height of 60 cm.

These values for heart rate (330 beats/min), aortic column height (85 cm) and atrial filling pressure (up to 30 cmH_2O) were therefore used in my initial studies to determine those perfusion conditions likely to produce maximum heart function (section 5.3).

The effects of different substrates or their combinations on

heart function in this and other models, has been incompletely studied by previous workers. Thus,although Scheuer and his colleagues have quantified the mechanical function of the isolated heart in considerable detail (section 2.3D), they have used only glucose, or glucose with added insulin, as their perfusion substrates. On the other hand, Neely and his colleagues[434,436-440], and others[441-443] have detailed the myocardial metabolic preferences and metabolic interactions during increased heart work, but have not related these biochemical events to myocardial mechanical function.

There is however some evidence that certain aspects of myocardial function are indeed substrate-dependent. Thus,myocardial oxygen consumption rates are greater in hearts perfused with free fatty acids than with glucose[434], and in this model[444,445], in intact animals[446,447] and in man[448,449] myocardial oxygen consumption rates increase as the free fatty acid concentration is increased. Lactate[450], pyruvate[451], acetoacetate[451] and β-hydroxybutyrate[451] also increase myocardial oxygen consumption rates. In contrast, others have reported that free fatty acids[452] and free fatty acid mixture[450] do not cause increased myocardial oxygen consumption rates. The higher myocardial oxygen consumption rates in dogs[446], in swine[447] and in man[449] following elevation of circulating free fatty acid levels, and in isolated hearts perfused with free fatty acids[444],are not due to increased heart work, suggesting that high levels of circulating free fatty acids impair myocardial efficiency thereby causing "oxygen wastage".

Myocardial mechanical performance has also been shown to be substrate-dependent. Thus,in the isolated papillary muscle preparation (section 2.3D) Henderson, Most, Parmley et al[453] found that,during fully oxygenated perfusions (normoxia),mechanical function measured as peak

developed force was not different between muscles perfused with either glucose, linoleic acid or with a combination of both substrates. However, during hypoxia or anoxia, glucose-perfused muscles showed a less rapid decline in peak developed force and a lesser rise in diastolic tensions than did muscles perfused with either glucose and linoleic acid, or with linoleic acid alone. Changes in peak developed force paralleled changes in the rates of force development, and generally reflected the perfusate fatty acid concentrations. During re-oxygenation, the extent of recovery was related to the severity of the mechanical depression that had occurred during anoxia or hypoxia, so that glucose-perfused muscles recovered better than did those perfused with free fatty acids.

Snow[454] has recently compared the effects of the perfusate substrates, butyrate and glucose on papillary muscle function during normoxia, hypoxia and during re-oxygenation. During normoxia, maximum developed tensions were slightly greater and times to peak tension slightly longer in butyrate-perfused muscles, but the rates of tension development were not different between groups. However, during hypoxia and subsequent re-oxygenation, mechanical function was, in agreement with the findings of Henderson and his colleagues, superior in glucose-perfused muscles.

In the isolated, non-working Langendorff preparation, hearts contracting isovolumically around a left ventricular balloon catheter had significantly greater diastolic pressures and significantly lower peak left ventricular pressures and rates of pressure development when perfused with high concentrations of free fatty acids (linoleic or octanoic acid) than with dextrose[445]. In direct contrast, Willebrands and van der Veen[450] reported that, during fully oxygenated perfusions, amplitudes of contraction (and coronary flow rates) were greatest in non-working hearts perfused with free fatty acid mixture; they were less in hearts perfused with either

lactate or pyruvate, and were lowest in glucose-perfused hearts. Also in this model, Challoner and Steinberg[444] found higher coronary flow rates in hearts perfused with free fatty acids than with glucose. Similarly, in isolated working rat hearts, Neely, Whitmer and Mochizuki[434] reported that hearts perfused with free fatty acids (palmitate) maintained higher heart rates and greater left ventricular pressures for longer periods, than did glucose-perfused hearts.

In intact animals, similarly conflicting results have been reported. Thus Mjøs[446] found that increased rates of myocardial free fatty acid uptake were not associated with changes in left ventricular pressures, in the maximum rates of left ventricular pressure development, in cardiac outputs or in heart rates. In contrast, Liedtke, Nellis and Neely[447] found that when circulating free fatty acid levels were increased by intralipid infusions, intact pig hearts developed significant reductions in aortic pressures, in left ventricular systolic pressures, and in calculated left ventricular work during both normoxia and during acute experimental ischaemia. But, in contrast to findings in isolated preparations and in the intact animal, increased levels of circulating free fatty acids have not been associated with increased coronary flow rates[446,447,449,452].

From this literature survey it is clear that neither the perfusion conditions nor the optimum substrate combinations to elicit maximum heart function in the isolated perfused working rat heart model have been established. I therefore undertook the studies described in this chapter.

C. The perfusion and recording apparatus.

The isolated perfused working rat heart model originally described by Neely, Liebermeister, Battersby and Morgan[433] and modified by Opie, Mansford and Owen[442] was used in all experiments. Figure 5.1 is a diagrammatic representation of the apparatus.

In this model, the left side of the heart is perfused via a cannula in the left atrium and the infused fluid traverses the mitral valve, thereby entering the left ventricle. Spontaneous or pacing-induced ventricular contractions then eject the fluid past the aortic valve via an aortic bubble trap into the aortic column. The bubble trap adds elastic compliance to the aortic column, the so-called Winkessel effect. During the period of Langendorff retrograde pre-perfusion (see below), this bubble trap also prevents bubbles contained in the perfusion fluid from entering the heart. The aortic column has a fixed diameter and height, thereby ensuring a constant aortic flow resistance.

The fluid leaving the left ventricle during contraction passes either into the aortic column, from where its overflow (the aortic output) can be measured in a calibrated chamber, or it passes into the coronary arteries thereby perfusing the coronary bed. Coronary arterial flow returns via the coronary sinus to the right atrium and thence to the right ventricle from where it is ejected through the pulmonary artery. As the right side of the heart is not cannulated in this preparation, the fluid ejected from the pulmonary artery drips over the apex of the heart into the heart perfusion jacket from where it may be collected and measured as the coronary flow rate. The sum of the rates of aortic output and coronary flow thus measured, equals the cardiac output corrected to either

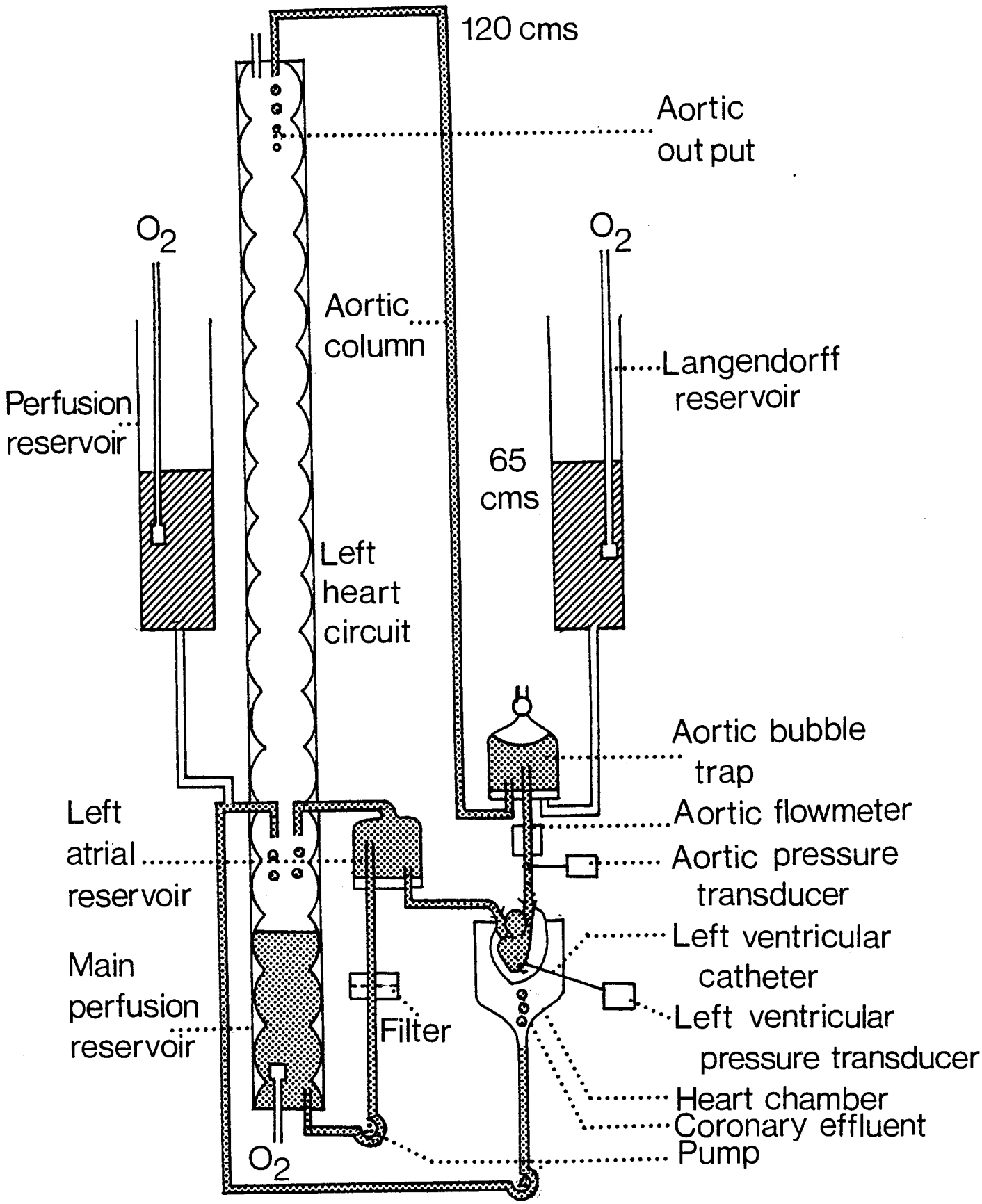

Figure 5·1

Diagram of the isolated perfused working rat heart model.
For details see text.

ml/min or ml/g left ventricular dry weight/min.

When coronary effluent is not being measured, it is recirculated by a pump (Marlow-Watson Limited, Falmouth, Cornwall) to the main perfusion reservoir where it is continually gassed with 95% oxygen and 5% carbon dioxide. The aortic overflow also enters this reservoir by dripping down the inside of the glassware that supports the aortic column and acts as the "lung".

Fully oxygenated fluid from this reservoir is continually pumped by the same Watson-Marlow pump through an Inline-47 Millipore filter unit (Millipore Corporation, Bedford, Massachusetts) containing two Millipore disc filters (pore sizes 5,0 and 0,8 μm) to the left atrial reservoir from where the fluid either enters the heart via the left atrial cannula or is returned to the main perfusion reservoir by an overflow escape mechanism. The temperature of the perfusion fluid is maintained at $37^{o}C$ by water heated and continually re-circulated in the outer jacket of the perfusion glassware, by a Thermomix pump (B. Braun, Melsungen, West Germany).

To ensure that differences in heart rates are not a factor influencing differences in mechanical performance, all hearts, except for those reported under section 5.3A, were paced for the entire duration of the experiments via a silver wire attached to the right atria. The pacing stimulus came from either a Grass S88 stimulator (Grass Instruments, Quincy, Massachusetts) or a custom-built model, both of which produced square pulse waves of 4 milliseconds duration at 4 millivolts.

In some experiments, aortic and left ventricular pressures were also measured. Aortic pressures were obtained from a side-arm of the aortic cannula located 4 cm above the aortic valve and connected to a

Statham P23 pressure transducer (Statham Instruments Company, Hato Hey, Puerto Rico). Recordings were obtained at 100 mm/sec on a Grass polygraph pen recorder, Model 79D (Grass Instruments, Quincy, Massachusetts). Left ventricular pressures were obtained from a 4 cm length PE-60 polyethylene catheter (Clay Adams, New York) which was connected through a wide bore adaptor to a similar transducer-recording system. The calibration procedures for the pressure recording apparatus are described in Appendix 1.E.

In those experiments described in section 6.5, the apparatus was modified to allow on-line computer analysis of aortic flows and left ventricular and aortic pressures.

Aortic and left ventricular pressures were measured through dual-channel electronic pressure transducers (LX0603 series Monolithic Pressure transducers - National Semiconductor Corporation, Santa Clara, California), the electronic circuitory of which was designed by Drs. Hughes and North of the Department of Biomedical Engineering, University of Cape Town. The output from the transducers provided 8 volts at a maximum pressure of 210 mmHg.

Aortic flows were measured with an electromagnetic flow probe (Statham Instruments, Oxnard, California) built into the aortic cannula 2 cm distal to the pressure side-arm. The probe diameter was 1,78 mm with a cross-sectional area of 249 mm^2. The flow preamplifier was made by EMI (Homebush, Australia).

The left ventricular and aortic pressures, and aortic flows were samples in real-time by a Data General MicroNova minicomputer and transmitted to a Data General Eclipse S130 mini-computer for further processing. The sampling rate was 233,4 Hz per channel, corresponding to 4,28msec between samples. Since the highest meaningful frequency of

the cardiac waveforms is 50 - 60 Hz, this sampling rate met the Nyquist criterion. The MicroNova 12-bit analogue to digital converter has a resolution of 1 part in 4096. However, less than half the converter's full range was used. Instantaneous pressures were measured approximately to the nearest 0,25 mmHg and aortic flows to the nearest 0,6 ml/min. As this is within the reproducibility characteristics of the probes, the accuracy of these measurements was limited by the sampling technique.

The procedures by which the aortic and left ventricular pressures, and aortic flows were calibrated,are described in Appendix 1.E.

D. Mounting the isolated rat heart.

Hearts from male Wistar-Weissman albino rats weighing between 230 and 280 grams were used in all experiments. Prior to removal of their hearts, each animal was weighed on a laboratory scale (Ohaus Scale Corporation, New Jersey).

Extreme care was taken to ensure that,when the heart was mounted onto the cannula, there were no bubbles in the aorta. Immediately the aorta had been cannulated, the heart was held in position with a small arterial clamp, and retrograde Langendorff perfusion was immediately commenced. During this retrograde perfusion, the perfusion pressure was maintained at 65 cmH_2O and the hearts were perfused with Krebs-Henseleit buffer (Appendix 1.B) containing 11,1 mM D glucose alone, gassed with 95% O_2:5% CO_2. Spontaneous cardiac activity resumed almost immediately this perfusion commenced.

The aorta was next secured to the cannula with surgical thread, care being taken to ensure that the tip of the aortic cannula neither penetrated the aortic valve, nor that a large area of aorta was left

between the tip of the aortic cannula and the aortic valve. This latter precaution was necessary to prevent aneurysmal dilatation of the aorta occurring during the perfusion period. Empirically it was also noted that aneurysmal aortic dilatation was more likely to occur if the aorta had been stretched by over-vigorous manipulation of the heart as it was excised from the chest.

After the aorta had been secured to its cannula, the tissues behind the heart were dissected off to expose the pulmonary veins, through one of which the atrial perfusion cannula was passed. At least 2 surgical ties were used to secure the left atrium to its cannula, and care was taken to ensure that all the pulmonary veins had been included in these ligatures. This was necessary to prevent left atrial "leaks" - that is left atrial fluid escaping through an unattached pulmonary vein, thereby falsely increasing the rate of measured coronary flow. Immediately prior to the cannulation of the pulmonary vein, the left atrial cannula was flushed of any air bubbles that might have been present.

In those experiments in which left ventricular pressures were to be measured, the tip of a 4 cm long left ventricular catheter was introduced, according to the technique described by Penpargkul and Scheuer[260], through the pulmonary vein into the left atrium from where it was carefully directed through the mitral valve, to pierce the apex of the heart. Left atrial cannulation was then completed in the conventional manner, and the left ventricular catheter was attached through a wide-bore adaptor to the pressure transducer. A flange produced at the proximal end of the ventricular catheter, by holding it over the end of a hot matchstick, prevented the catheter from slipping through the left ventricular wall.

Complete mounting of the heart together with left ventricular catheterization was usually completed within 10 minutes. Five minutes

later, that is approximately 15 minutes after removal of the heart from the rat, this pre-perfusion period ended, and working heart perfusion was commenced by allowing fluid to flow into the left atrium. The tap on the tubing leading from the Langendorff perfusion column was then closed, and the tap on the aortic column opened.

E. The perfusion fluid.

The perfusion fluid used in all experiments was a modified Krebs-Henseleit buffer to which a variety of substrates, hormones, or the drug isoproterenol (Isuprel$^{(R)}$ - Winthrop Laboratories) were added when appropriate for the particular experimental protocol. These details are described in Appendix 1.B.

F. Perfusion fluid analyses.

In those experiments in which myocardial metabolism was studied, the perfusion fluid was analyzed, according to the methods described in Appendix 1.C, for some or all of the following: perfusate glucose, lactate, pyruvate, free fatty acid, ^{3}H-sorbitol and free calcium concentrations, oxygen tensions, and coronary effluent $^{3}H_2O$ contents.

G. Tissue biochemical analyses.

In those experiments which were terminated by clamping the hearts in pre-cooled Wollenberger tongs, the hearts were stored and later analyzed, for some or all of the following: ATP, PCr, glycogen, citrate, lactate, cyclic AMP and cyclic GMP concentrations and dry heart weights, according to the methods described in Appendix 1.D.

The decision to express all data relative to left ventricular, not whole heart weight, was taken because it was felt that correction of, particularly, heart performance data (cardiac output and stroke volume) to whole heart weight would underestimate the true contribution of the left ventricle. Errors produced by this method of data presentation are that myocardial oxygen consumption rates, tissue metabolite levels and rates of myocardial glycolysis are probably overestimated if only the left ventricle is considered to be metabolically-active or to contain metabolites. It was felt that this error would be quite small as even though right ventricular weights may be as much as 25% of left ventricular weight, during working, left atrial perfusion, the contribution of the right ventricle to overall metabolism is likely to be quite small.

At the termination of each of those experiments studying the effects of exercise training on actomyosin and myosin ATPase activities, and on the extent of troponin-1 and myosin P light chain phosphorylation (section 6.6B), hearts were rapidly removed from the perfusion apparatus, homogenized and prepared for analysis according to the methods also described in Appendix 1.D.

H. Calculation and expression of results and statistical methods.

These are fully described in Appendix 1.F.

5.3 STUDIES OF MAXIMUM HEART WORK IN THE ISOLATED PERFUSED RAT HEART MODEL.

A. Comparison of mechanical function of hearts perfused with either 11,1 mM D(+)-glucose or with 11,1 mM D(+)-glucose plus 10 mM DL β-OH butyrate under conditions of increasing atrial filling pressure.

This initial study was designed principally as a screening procedure to establish roughly those conditions of preload (atrial filling pressure) and aortic column height (pressure head) that elicited maximum heart function in this model, and to determine whether the perfusion substrate content influenced this maximum.

Experimental protocol.

Unpaced, isolated working rat hearts were perfused with either 11,1 mM D(+)-glucose or 11,1 mM D(+)-glucose plus 10 mM DL β-OH butyrate for 20 minutes at each of the following atrial filling pressures: 10, 15, 20 and 25 cmH_2O against an aortic column height of 90 cm; these conditions approximating those originally used by Penpargkul and Scheuer[260].

Rates of coronary flow and aortic outputs were measured every 5 minutes by collecting timed samples of perfusate in a graduated cylinder and correcting the result to give the value in ml/min. Cardiac outputs were calculated as the sum of aortic outputs and coronary flow rates corrected for left ventricular dry weight. Heart rates were determined from the aortic pressure traces, and stroke volumes were calculated as cardiac outputs divided by heart rate. Perfusate oxygen tensions were measured every 20 minutes, from which data rates of myocardial oxygen consumption were calculated (Appendix 1.F).

Results and discussion.

The results of this group of experiments are depicted in Figure 5.2.

It can be seen that,at atrial filling pressures of 20 and 25 cmH_2O,cardiac outputs were significantly greater in hearts perfused with 11,1 mM glucose plus 10 mM β-OH butyrate than they were in hearts perfused with 11,1 mM glucose alone. Furthermore, in hearts perfused with glucose plus β-OH butyrate, cardiac outputs, heart rates and rates of myocardial oxygen consumption were all highest at atrial filling pressures of 25 cmH_2O implying that,in the presence of adequate substrate, peak mechanical

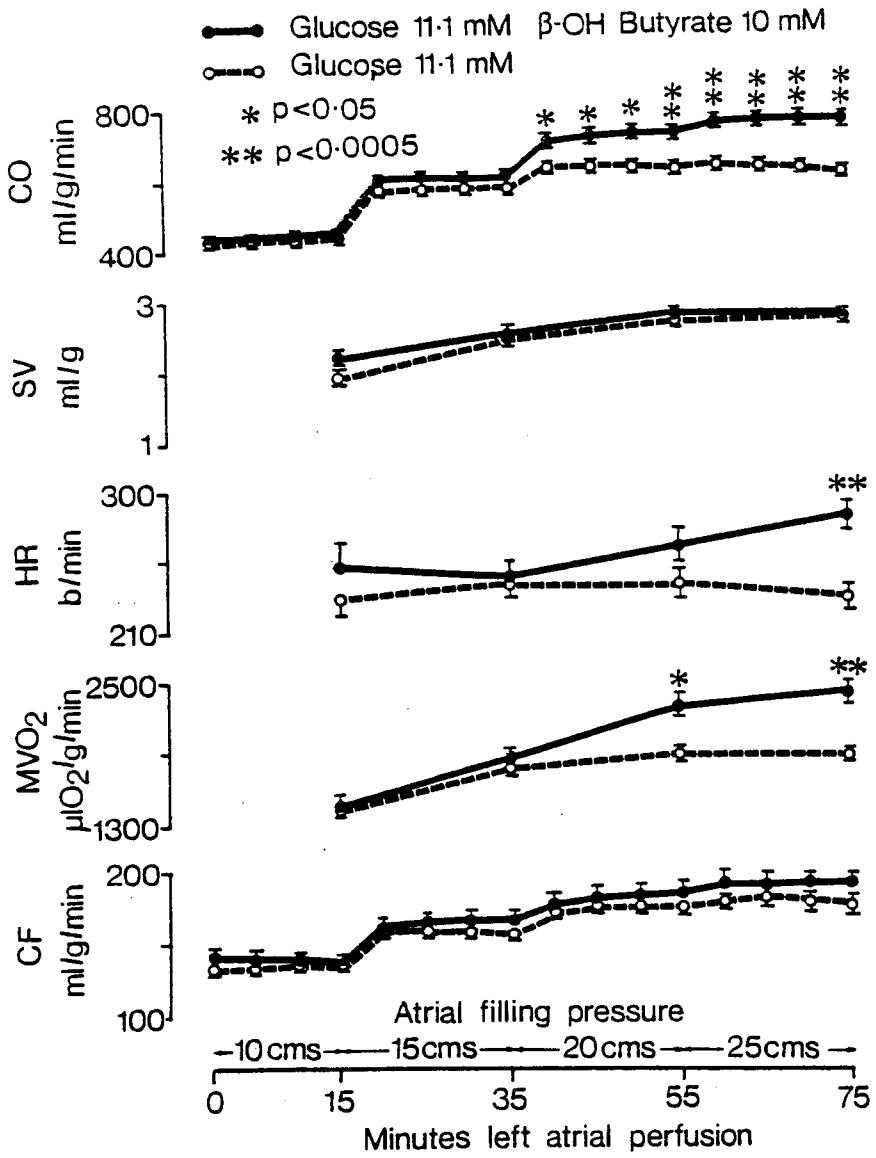

Figure 5.2

Comparison of mechanical function of hearts perfused with either glucose or with glucose plus β-OH butyrate under conditions of increasing atrial filling pressure.
Note that hearts perfused with glucose plus β-OH butyrate have higher cardiac outputs, heart rates and myocardial oxygen consumption rates at the higher atrial filling pressures.
For abbreviations please turn over.

Abbreviations:

CO	-	Cardiac output
SV	-	Stroke volume
HR	-	Heart rate
$M\dot{V}O_2$	-	Myocardial oxygen consumption
CF	-	Coronary flow rate

Each point on the graph indicates Mean ± SEM for 11 measurements.

function in the isolated perfused rat heart model is not elicited by atrial filling pressures below 25 cmH_2O.

Stroke volumes and coronary flow rates were not significantly different between hearts perfused with either substrate combination. Thus, the significantly greater cardiac outputs of hearts perfused with the glucose/β-OH butyrate combination resulted from their ability to increase heart rates whilst maintaining constant stroke volumes at the higher atrial filling pressures.

B. Studies to determine which substrate combination produces maximum heart work, and the metabolic and mechanical reasons therefor.

Effects of different substrate combinations on overall heart function.

Experimental protocol.

The results of the previous preliminary study clearly showed that maximum cardiac output, and therefore peak heart function, were not achieved in isolated hearts perfused at atrial filling pressures below 25 cmH_2O. Thus, in the series of experiments reported in this section, hearts were perfused at a constant atrial filling pressure of 25 cmH_2O for the entire duration of the experiments. To increase further the workload on the heart, the aortic column height was empirically increased to 1,2 metres. As will be shown in the studies reported in subsequent sections of this chapter, these perfusion conditions fortuitously elicited peak heart function, judged as maximum cardiac outputs and calculated heart work, because further elevation of the atrial filling pressure to 30 cmH_2O

caused either a fall or no further increase in either of these parameters.

For these experiments the perfusion protocol was as follows: After an initial 15-minute Langendorff pre-perfusion period, working hearts paced at 330 beats/min were perfused for 65 minutes at an atrial filling pressure of 25 cmH_2O with the aortic column height constant at 1,2 metres. Measurements of aortic outputs, rates of both coronary flow and myocardial oxygen consumption were taken every 10 minutes and considered to be representative of heart function during that 10-minute period. After 65 minutes' left atrial perfusion, the experiments were terminated either by clamping the hearts in Wollenberger tongs or by preparing the hearts for the determination of left ventricular dry weights. Frozen hearts were then prepared and extracted for biochemical analysis according to methods described in Appendix 1.D. To estimate the left ventricular fresh and dry weights and right ventricular dry weights of those hearts clamped for biochemical studies, and which could therefore not be weighed, the following linear regression equations were generated from 36 experiments in which body weights, left ventricular dry and fresh weights, and right ventricular dry weights were measured:

L.V. fresh weight (g) = 0,002 (body weight g) + 0,01, where r^2 = 0,83.

L.V. dry weight (g) = 0,0003 (body weight g) + 0,03 where r^2 = 0,95.

R.V. dry weight (g) = 0,00007 (body weight g) + 0,01 where r^2 = 0,67.

Hearts were perfused with a wide variety of perfusion substrates made up essentially of different combinations and concentrations of glucose, lactate, pyruvate, β-OH butyrate, palmitate/albumin, to some of which insulin had been added.

Results and discussion

Tables 5.1 to 5.3 record the mean cardiac outputs and mean rates of coronary flow and myocardial oxygen consumption after 30 minutes' left

TABLE 5.1

STROKE VOLUMES AND RATES OF CORONARY FLOW AND MYOCARDIAL OXYGEN CONSUMPTION AFTER 30 MINUTES STEADY STATE PERFUSIONS WITH DIFFERENT SUBSTRATE COMBINATIONS.

PART 1: COMPARISON AMONGST HEARTS PERFUSED WITH DIFFERENT COMBINATIONS AND CONCENTRATIONS OF GLUCOSE, LACTATE, PYRUVATE AND INSULIN.

SUBSTRATE COMBINATION	STROKE VOLUMES ml/g	MYOCARDIAL OXYGEN CONSUMPTION RATES µℓ O_2/g/min	CORONARY FLOW RATES ml/g/min
11,1 mM Glucose	2,1 ±0,1 (6)	2065,1 ±72,5 (6)	196,0 ±8,6 (6)
10 mM Lactate	2,3 ±0,1 (6)	2467,6 ±110,5 (6)	229,4 ±5,7 (6)
11,1 mM Glucose 10 mM Lactate	2,2 ±0,1 (6)	2467,0 ±74,2 (6)	208,2 ±5,8 (6)
11,1 mM Glucose 4,5 mM Lactate Insulin 2 U/L	2,1 ±0,1 (6)	2401,7 ±129,8 (6)	204,6 ±8,9 (6)
11,1 mM Glucose 6,6 mM Lactate Insulin 2 U/L	2,1 ±0,1 (6)	2363,5 ±111,7 (6)	197,8 ±5,7 (6)
11,1 mM Glucose 13,2 mM Lactate Insulin 2 U/L	2,2 ±0,1 (6)	2472,6 ±70,8 (6)	222,1 ±4,4 (6)
11,1 mM Glucose 10 mM Pyruvate Insulin 2 U/L	2,1 ±0,1 (6)	2091,1 ±73,7 (6)	194,1 ±9,4 (6)
11,1 mM Glucose 10 mM Lactate Insulin 2 U/L	2,5 ±0,1 (6)	2846,2 ±99,8 (6)	222,7 ±6,7 (6)
11,1 mM Glucose Insulin 2 U/L	2,6 ±0,1 (6)	2493,2 ±143,5 (6)	230,6 ±8,7 (6)

Note that stroke volumes and coronary flow rates are highest in hearts perfused with either 11,1 mM glucose plus insulin or with 11,1 mM glucose plus 10 mM lactate plus insulin. Higher or lower lactate concentrations caused reduced stroke volumes.
Values are Mean ± SEM (number of measurements).

TABLE 5.2

STROKE VOLUMES AND RATES OF CORONARY FLOW AND MYOCARDIAL OXYGEN CONSUMPTION AFTER 30 MINUTES STEADY STATE PERFUSIONS WITH DIFFERENT SUBSTRATE COMBINATIONS.

PART 2. EFFECTS OF ADDING GLUCOSE-INSULIN TO PALMITATE, AND OF INCREASING THE PERFUSATE FREE FATTY ACID CONCENTRATION.

SUBSTRATE COMBINATION	STROKE VOLUMES ml/g	MYOCARDIAL OXYGEN CONSUMPTION RATES $\mu/O_2/g/min$	CORONARY FLOW RATES ml/g/min
1 mM Palmitate (3% Albumin)	1,8 ±0,1 (6)	2170,0 ±134,7 (6)	204,7 ±8,4 (6)
1 mM Palmitate (3% Albumin) 11,1 mM Glucose Insulin 2 U/L	2,1 ±0,1 (6)	2301,1 ±139,6 (6)	224,4 ±11,1 (6)
1,5 mM Palmitate (1,5% Albumin) 11,1 mM Glucose Insulin 2 U/L	1,5 ±0,1 (6)	2016,0 ±156,8 (6)	219,0 ±8,3 (6)

Note that the addition of glucose-insulin to palmitate increases stroke volumes, but that increasing the perfusate free fatty acid concentration beyond 1 mM and reducing the perfusate albumin concentration, causes stroke volumes to fall.

Values are Mean ± SEM (number of measurements).

TABLE 5.3

STROKE VOLUMES AND RATES OF CORONARY FLOW AND MYOCARDIAL OXYGEN CONSUMPTION AFTER 30 MINUTES STEADY STATE PERFUSIONS WITH DIFFERENT SUBSTRATE COMBINATIONS.

PART 3. EFFECTS OF ADDITION OF KETONE BODIES

SUBSTRATE COMBINATION	STROKE VOLUMES ml/g	RATES OF MYOCARDIAL OXYGEN CONSUMPTION $\mu/O_2/g/min$	CORONARY FLOW RATES ml/g/min
11,1 mM Glucose	2,2	2292,2	214,6
10 mM B-OH Butyrate	0,1	110,3	4,0
	(6)	(6)	(6)
11,1 mM Glucose	2,3	2130,9	212,7
10 mM B-OH Butyrate	0,1	71,1	4,6
Insulin 2 U/L	(6)	(6)	(6)
11,1 mM Glucose	2,0	2189,4	191,1
10 mM Lactate	0,1	152,5	8,7
10 mM B-OH Butyrate	(6)	(6)	(6)
Insulin 2 U/L			

Note that the addition of 10 mM B-OH Butyrate to the optimum 11,1 mM glucose-10 mM lactate-insulin combination (Table 5.1) caused stroke volume to fall.

Values are Mean ± SEM (number of measurements).

atrial perfusion, for hearts perfused with the different substrate combinations. Only the 30-minute values are reported, as they were representative of the findings for the entire perfusion period. By tabulating only the data collected at that time, interpretation is greatly simplified.

There were 3 essential findings from these experiments. First, stroke volumes were greatest in hearts perfused with either 11,1 mM glucose plus insulin or with 11,1 mM glucose and 10 mM lactate plus insulin (Table 5.1). Higher (13,5 mM) or lower (6,6 or 4,5 mM) lactate concentrations were associated with lower stroke volumes when combined with glucose/insulin. Similarly, increasing the free fatty acid concentration to 1,5 mM and reducing the albumin concentration, thereby further increasing the FFA/albumin molar ratio (Table 5.2), or adding 10 mM β-OH butyrate to the optimum glucose/lactate/insulin solution, resulted in reduced stroke volumes (Table 5.3). Thus, it is clear that the addition of excess substrate to the perfusate reduces, rather than increases, stroke volumes.

Second, hearts perfused with fatty acid solutions had uniformly lower stroke volumes than did hearts perfused with non-fatty acid substrates.

But the third and probably the most striking finding is presented in graphical form in Figure 5.3. This graph shows that stroke volumes were not different in hearts perfused with either 11,1 mM glucose or with 11,1 mM glucose and 10 mM pyruvate plus insulin at 2 units/litre, whereas hearts perfused with 11,1 mM glucose plus insulin had significantly increased stroke volumes and coronary flow rates. Thus, whereas the addition to the perfusate of insulin (or lactate plus insulin - Table 5.1) to glucose, significantly increased stroke volumes and coronary flow rates, the addition of 10 mM pyruvate with insulin to glucose, failed to increase either stroke volumes, or rates of coronary flow.

To study these findings further, I next chose to compare the

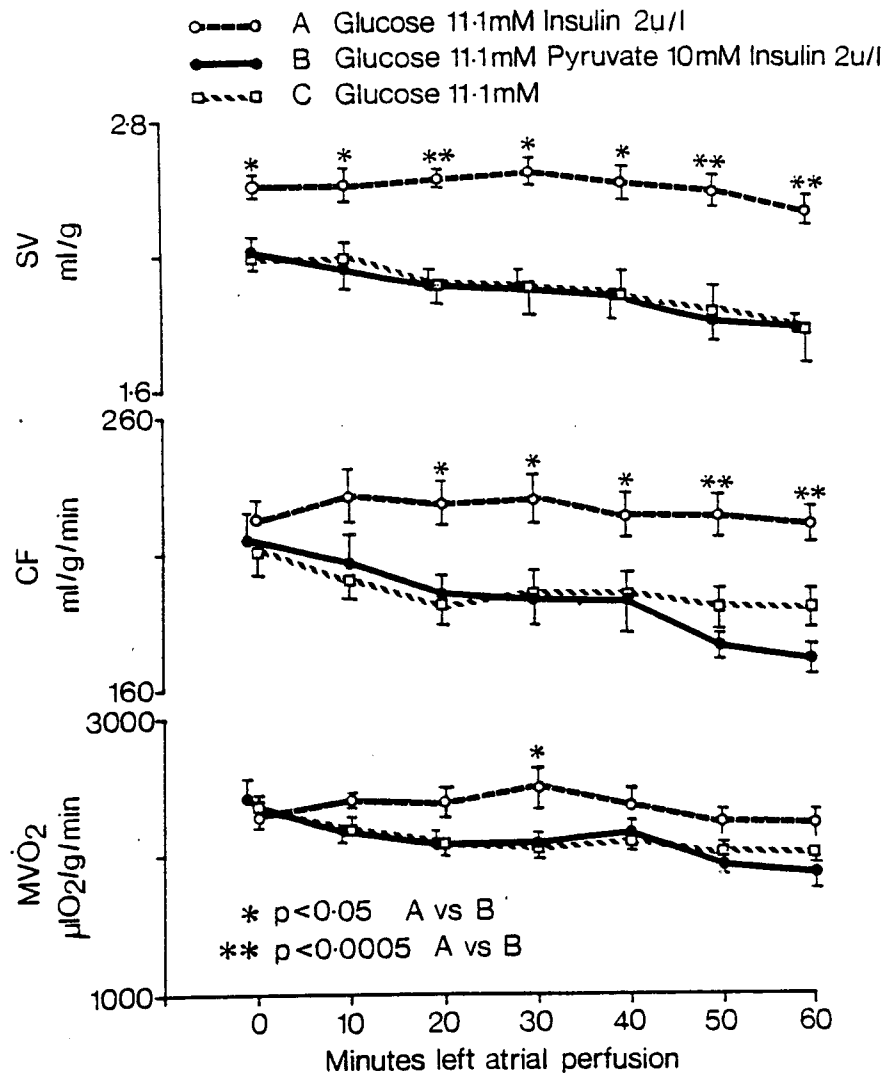

Figure 5.3

Effect of changing substrate from glucose to glucose-insulin, to glucose - insulin - pyruvate on mechanical performance of working rat hearts.

Note that hearts perfused with glucose-insulin have the highest stroke volumes and coronary flow rates.

For abbreviations please turn over.

Abbreviations.

SV - Stroke volume
CF - Coronary flow rate
$M\dot{V}O_2$ - Myocardial oxygen consumption

Each point represents Mean ± SEM for 6 observations.

metabolism of hearts perfused with these different substrate combinations, and the effects of these substrate combinations on left ventricular function.

Effects of different substrate combinations on myocardial metabolism.

Experimental protocols.

To compare the effects of the different substrate combinations on myocardial metabolism, isolated hearts were mounted on the perfusion apparatus in the conventional manner, and after an initial 15-minute non-working,Langendorff perfusion, left atrial perfusion commenced. Hearts were then perfused in a recirculating system for a further 70 minutes at a constant atrial filling pressure of 25 cmH_2O, at a heart rate of 330 beats/min and with a constant aortic column height of 120 cm. In all experiments, the volume of recirculating fluid was approximately 100 ml.

Following an initial 5-minute working heart equilibration period, 5 ml aliquots of perfusion fluid were samples for the initial concentrations of perfusion substrate (S_5), the background sorbitol concentrations (H_5) and, in experiments in which lactate was not a perfusion substrate, the initial lactate concentrations (L_5). The system was now considered "closed" and every effort was made to prevent the loss of fluid from the system.

After 35 minutes' perfusion, 50 µl of H^3-sorbitol diluted 1:1 in distilled water was added for the subsequent calculation of the circulating volume as described in Appendix 1.F. Sixty five minutes after left atrial perfusion had commenced, additional 5 ml aliquots of perfusion fluid were samples for the measurement of the final substrate (S_{65}),

sorbitol (H_{65}) and lactate (L_{65}) concentrations. Five minutes thereafter, hearts were clamped in pre-cooled Wollenberger tongs and prepared and stored for later determination of myocardial ATP, PCr, glycogen and citrate levels, according to the methods described in Appendix 1.D. Perfusion fluids were analysed within 24 hours according to the methods described in Appendix 1.C.

The perfusate substrate combinations used in these experiments were similar to those used in the experiments reported in the previous section and are listed in Tables 5.4 and 5.6. One series of 9 hearts was perfused without any substrate being added to the perfusion fluid. These hearts were unable to maintain aortic outputs for more than 15 minutes left atrial perfusion, at which time these experiments were terminated.

Another series of 6 hearts was clamped with Wollenberger tongs after the initial 15 minutes Langendorff pre-perfusion, and the tissue extracted for the determination of myocardial ATP, PCr, glycogen, citrate and cyclic AMP levels. The mean glycogen value measured in these hearts was taken as the initial (G_o) glycogen level present in all hearts immediately left atrial perfusion commenced, and was used in the calculation of rates of glycogenolysis and glycolysis (Appendix 1.F).

The data from these experiments were used to calculate the following for each substrate combination, according to the methods described in Appendices 1.D and 1.F:

substrate uptake rates (μmol/g/min),

rates of glycolytic flux (μmol glucose equiv./g/min),

rates of glycolytic ATP production (μmol/g/min)

rates of lactate release (μmol/g/min),and

rates of C6 oxidation (μmol/g/min).

Results and discussion.

Myocardial ATP, PCr, glycogen and citrate levels after 70 minutes left atrial perfusion with the different substrate combinations.

Myocardial levels of ATP, PCr, glycogen and citrate after 70 minutes' left atrial perfusion with the different substrates are listed in Table 5.4.

Tissue ATP levels were highest in hearts perfused with glucose-insulin, in which group myocardial ATP levels were not different from values measured in hearts after 15 minutes Langendorff non-working heart perfusions (Table 5.5). These values were higher than values for hearts perfused with either glucose, lactate, palmitate or palmitate-glucose-insulin but are not significantly different from values for hearts perfused with either glucose-pyruvate-insulin or glucose-lactate-insulin. Therefore, the addition of glucose-insulin to all perfusion substrates, except palmitate, increased tissue ATP levels measured after 70 minutes left atrial perfusion.

PCr values were highest in hearts perfused with the non-glucose substrates (lactate, pyruvate, palmitate) but all values were lower than those measured in hearts perfused for 15 minutes in the Langendorff mode. The addition of glucose-insulin to all perfusion substrates increased tissue PCr levels, which were highest in pyruvate-glucose-insulin hearts.

The addition of insulin to glucose and of glucose-insulin to lactate and palmitate increased terminal glycogen levels, which were highest in pyruvate-glucose-insulin perfused hearts. The lowest glycogen values occurred in hearts perfused without substrate for 15 minutes left atrial perfusion.

TABLE 5.4

MYOCARDIAL LEVELS OF ATP, PCr, GLYCOGEN AND CITRATE AFTER 70 MINUTES' LEFT ATRIAL PERFUSION WITH DIFFERENT SUBSTRATE COMBINATIONS.

SUBSTRATE COMBINATION	ATP µmol/g	PCr µmol/g	GLYCOGEN µmol glucose equiv/g	CITRATE µmol/g
A. 1 mM Palmitate (3% Albumin)	2,42 ±0,22 (13)	4,78 ±0,52 (13)	9,60 ±0,60 (13)	0,57 ±0,05 (13)
B. 10 mM Lactate	2,87 ±0,26 (15)	4,54 ±0,40 (15)	9.07 ±0,55 (15)	0,36 ±0,03 (15)
C. 10 mM Pyruvate 11,1 mM Glucose Insulin 2 U/L	3,48 ±0,11 (11)	4,98* ±0,16 (11)	14,03 ±1,13 (11)	1,32** ±0,06 (11)
D. 1 mM Palmitate (3% Albumin) 11,1 mM Glucose Insulin 2 U/L	2,80 ±0,18 (14)	4,49 ±0,24 (14)	12,54 ±1,26 (14)	0,64 ±0,05 (14)
E. 11,1 mM Glucose	2,84 ±0,10 (19)	3,83 ±0,31 (18)	7,75 ±0,94 (20)	0,19 ±0,04 (16)
F. 10 mM Lactate 11,1 mM Glucose Insulin 2 U/L	3,32 ±0,18 (12)	4,76 ±0,18 (12)	12,35 ±1,22 (12)	0,64 ±0,06 (12)
G. 11,1 mM Glucose Insulin 2 U/L	3,64 ±0,11 (12)	4,13 ±0,27 (12)	10,93+ ±1,5 (13)	0,28++ ±0,03 (13)
H. No substrate	3,23 ±0,25 (9)	3,76 ±0,65 (9)	6,21 ±0,59 (9)	0,09 ±0,01 (6)

Values are Mean ± SEM (number of measurements).

* $P < 0,02$ for comparison between C and G.
** $P < 0,05 \times 10^{-7}$ for comparison between C and G.
\+ $P < 0,01$ for comparison between G and H.
++ $P < 0,05 \times 10^{-3}$ for comparison between G and H.

TABLE 5.5

MYOCARDIAL LEVELS OF ATP, PCr, GLYCOGEN, CITRATE AND CYCLIC AMP AFTER 15 MINUTES' LANGENDORFF PERFUSION, AFTER AN ADDITIONAL 15 MINUTES' LEFT ATRIAL PERFUSION, AND AFTER A FURTHER 25 MINUTES' LEFT ATRIAL PERFUSION DURING WHICH $6,5 \times 10^{-7}$M ISOPROTERENOL WAS INFUSED.

	PERFUSION CONDITIONS	ATP µmol/g	PCr µmol/g	GLYCOGEN µmol glucose equiv/g	CITRATE µmol/g	cAMP nmol/g
A.	15 min Langendorff perfusion	3,61 ±0,25 (9)	6,06 ±0,30 (8)	18,18 ±1,69 (9)	0,21 ±0,02 (4)	-
B.	15 min subsequent left atrial perfusion	3,53 ±0,20 (6)	3,64** ±0,25 (6)	14,37 ±0,57 (7)	0,33* ±0,03 (6)	0,48 ±0,06 (7)
C.	25 min subsequent left atrial perfusion with isoproterenol	3,16 ±0,17 (7)	4,21 ±0,21 (7)	7,17++ ±0,57 (8)	0,13+ ±0,02 (7)	0,84+ ±0,04 (8)

Note that PCr levels fall significantly during working heart perfusions, and that isoproterenol infusion causes tissue glycogen and citrate levels to fall, and cyclic AMP levels to rise significantly. All values are Mean ± SEM (number of measurements). The perfusion substrate supplied to Langendorff-perfused hearts was 11,1 mM D(+) glucose. All other hearts were perfused with 11,1 mM D(+) glucose plus insulin at 2 U/litre. $6,5 \times 10^{-7}$M isoproterenol was added at a constant infusion rate for 25 minutes, following 15 minutes control left atrial perfusions (section 5.3B).

* $p < 0,02$ for comparison between B and A.
** $p < 0,0001$ for comparison between B and A.
\+ $p < 0,0002$ for comparison between C and B.
++ $p < 0,05 \times 10^{-3}$ for comparison between C and B.

Myocardial citrate levels were higher in hearts perfused with non-glucose than with glucose substrates, and the addition of glucose-insulin to palmitate- or lactate-perfused hearts further increased these levels. Citrate levels were lowest in hearts perfused without substrate and were highest in hearts perfused with pyruvate-glucose-insulin, data which are in accord with the findings of Neely and his colleagues[434,440].

Thus, differences in tissue ATP, PCr, glycogen and citrate levels could not easily explain differences in mechanical function between hearts perfused with either glucose-insulin or glucose-pyruvate-insulin because, although ATP and glycogen levels were not different, both PCr and citrate levels were significantly lower in hearts perfused with glucose-insulin, in which hearts mechanical function was significantly better (Fig. 5.3).

It should also be noted that ATP and PCr levels were not different between hearts perfused either without substrate or with glucose-insulin. Thus, the inability of the former groups of hearts to maintain cardiac outputs for more than 15 minutes left atrial perfusion cannot be explained on the basis of the tissue high energy phosphate levels nor tissue glycogen levels which were similar to values measured in glucose-perfused hearts. It should be noted, however, that tissue citrate levels were very low in these hearts (Table 5.4).

Substrate metabolism of hearts perfused with the different substrate combinations.

Table 5.6 lists the myocardial rates of substrate uptake, of glycogenolysis, of glycolytic flux, of glycolytic ATP production, of

TABLE 5.6

SUBSTRATE METABOLISM OF HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS.

SUBSTRATE COMBINATION	SUBSTRATE UPTAKE µmol/g/min	GLYCOGEN UTILIZATION µmol glucose equiv/g/min	GLYCOLYTIC FLUX µmol glucose equiv/g/min	LACTATE RELEASE µmol/g/min	C6 OXIDATION µmol/g/min	GLYCOLYTIC ATP PRODUCTION µmol/g/min
1 mM Palmitate (3% Albumin)	0,86 ±0,01 (8)	0,12 ±0,01 (7)	0,12 ±0,01 (7)	0,65 ±0,12 (6)	-0,21 ±0,07 (5)	0,36 ±0,04 (7)
10 mM Lactate	7,72 ±0,62 (7)	0.16 ±0,01 (9)	0,16 ±0,01 (9)	-	0,16 ±0,01 (9)	0,48 ±0,03 (9)
10 mM Pyruvate 11,1 mM Glucose Insulin 2 U/L	Pyruvate 13,25 ± 1,13 (6) Glucose 1,69 ± 0,21 (6)	0,06 ±0,02 (6)	1,75 ±0,21 (6)	3,04 ±0,37 (6)	0,23 ±0,22 (6)	3,58 ±0,42 (6)
1 mM Palmitate (3% Albumin) 11,1 mM Glucose Insulin 2 U/L	Palmitate 0,89 ± 0,10 (9) Glucose 2,90 ± 0,51 (8)	0,11 ±0,03 (8)	3,00 ±0,53 (8)	0,89 ±0,10 (9)	2,12 ±0,56 (9)	6,12 ±1,07 (8)
11,1 mM Glucose	4,47 ±0,67 (7)	0,19 ±0,01 (7)	4,66 ±0,66 (7)	0,82 ±0,11 (7)	4,25 ±0,63 (7)	9,52 ±1,31 (7)
10 mM Lactate 11,1 mM Glucose Insulin 2 U/L	Lactate 6,16 ± 0,58 (7) Glucose 4,35 ± 0,56 (7)	0,07 ±0,03 (6)	4,66 ±0,63 (6)	-	4,66 ±0,63 (7)	9,38 ±1,25 (6)
11,1 mM Glucose Insulin 2 U/L	6,24 ±0,40 (9)	0,14 ±0,04 (7)	6,36 ±0,51 (7)	2,20 ±0,38 (9)	5,37 ±0,57 (7)	12,86 ±1,02 (7)

Hearts were perfused with the different substrate(s) for 70 minutes under steady state conditions (heart rates 330 beats/min, atrial filling pressure 25 cm H_2O, aortic column height 120 cms).

Note the effects of the different substrates on rates of glycolytic ATP production. In general, the highest rates are found in hearts with the highest stroke volumes (Tables 5.1 and 5.2).

It is not clear why palmitate perfused hearts had negative C_6 oxidation rates. This was also found in a subsequent series of similar experiments (Table 6.3).

Values are mean ± SEM (number of measurements).

lactate release and of C6 oxidation during steady state perfusions with the different substrate combinations.

It will be seen that the addition of glucose-insulin to the basic perfusion substrates had 5 principal metabolic effects:

(i) It increased rates of myocardial oxygen consumption, explicable on the basis of either increased heart work (Table 5.1) or of increased myocardial efficiency.

(ii) It decreased the rates of myocardial glycogen utilization.

(iii) It increased the rates of glucose uptake, glycolytic ATP production and C6 oxidation.

(iv) It increased the rates of myocardial lactate release.

(v) It did not alter the rates of uptake of the other substrate (lactate or palmitate) present in the perfusion medium.

This latter finding, that lactate or palmitate uptakes were not different between hearts perfused with either lactate or lactate-glucose-insulin or with palmitate or palmitate-glucose-insulin, is particularly significant because it indicates that the greater stroke volumes in the latter groups must be due to a specific "glucose-insulin" effect. However, in the presence of pyruvate, this "glucose-insulin" effect on stroke volume does not appear to be particularly effective because, as shown in Figure 5.3, stroke volumes were significantly lower in hearts perfused with glucose-pyruvate-insulin than they were in either glucose-insulin or glucose-lactate-insulin perfused hearts (Table 5.1) and they were not different from values in glucose-perfused hearts. No series of hearts was perfused with pyruvate alone because such a series would not have added to the argument that follows.

This "glucose-insulin" effect can theoretically be explained only on the basis of increased production of components or products of

glycolysis, because the only common metabolic pathway activated by the addition of glucose-insulin to the primary substrate combination is glycolysis (Fig. 5.4). Thus, it should be noted in Table 5.6 that rates of glycolytic ATP production are, in general, highest in those hearts with the highest stroke volumes (Tables 5.1 and 5.2).

Effects of different substrate combinations on left ventricular function.

In order to understand why the different substrate combinations had such markedly different effects on stroke volume, I considered it essential that left ventricular function should be measured in hearts perfused with a selected group of those substrate combinations studied in the previous section. To do this, it was necessary to introduce a catheter into the left ventricle as described in section 5.2D.

The perfusion protocol used in these studies was as follows: After a 15-minute Langendorff pre-perfusion, left atrial perfusion commenced. Hearts were perfused for 6 minutes at each of the following 8 combinations of atrial filling pressures and heart rates:

(i) Heart rates constant at 330 beats/min with successive atrial filling pressures of 15, 20 and 25 cmH_2O.

(ii) Atrial filling pressure constant at 25 cmH_2O with successive heart rates of 300, 360 and 390 beats/min.

(iii) Heart rates and atrial filling pressures constant at 330 beats/min and 30 cmH_2O respectively.

At each successive workload, aortic outputs and rates of both coronary flow and myocardial oxygen consumption were measured, and high-speed pressure trace recordings were made according to the methods des-

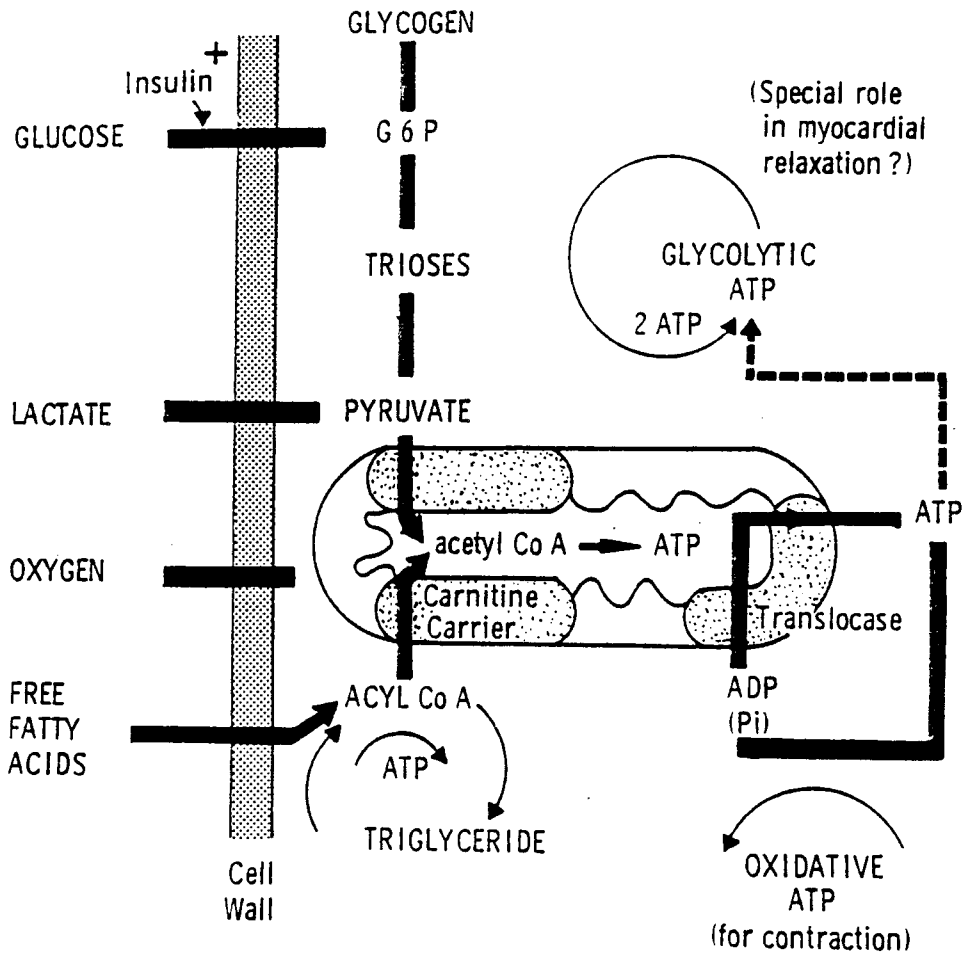

Figure 5·4

Substrate supply of the normal heart. Simplified flow diagram showing that the addition of glucose-insulin to lactate-, pyruvate-, palmitate- or glucose-perfused hearts activates glycolysis, which is an additional source of ATP production. It is suggested that this ATP source plays a special role in myocardial function.

cribed in section 5.2C. To measure left ventricular pressure changes during isovolumic contractions, pressure trace recordings were made at each workload, whilst the aortic tubing was clamped with surgical forceps for between 4-6 consecutive heart beats. Each new experimental condition was maintained for 3 minutes before data were collected.

At the end of the experiment, left ventricular dry weights were calculated, the left ventricular pressure traces analysed and cardiac output, stroke volume, heart work, myocardial efficiency and rates of both coronary flow and myocardial oxygen consumption were calculated according to the methods described in Appendix 1.F.

The complete results of all these studies are contained in Appendices 2.1 - 2.10. Table 5.7 presents this data in a more digestible form, as data from the particular perfusion condition producing peak heart function (heart rate 330 beats/min; atrial filling pressure 25 cmH_2O) are compared for the different substrate combinations.

The principal findings of these studies will be discussed in detail under the following headings:

(i) Effects on left ventricular function of the addition of insulin and glucose to the primary substrates - lactate, pyruvate, glucose and palmitate.

(ii) Comparison of left ventricular function in hearts perfused with either glucose, glucose-insulin, or glucose-pyruvate-insulin.

(iii) Evidence that the perfusion conditions used in section 5.3B produced maximum heart function.

There were 3 additional findings made during these studies. Although they do not have <u>direct</u> bearing on the main subject of this thesis, they are nevertheless included here, as they are relevant to the working rat heart model. These findings

TABLE 5.7

EFFECTS OF THE DIFFERENT SUBSTRATE COMBINATIONS ON LEFT VENTRICULAR FUNCTION AT ATRIAL FILLING PRESSURES OF 25 CM H2O AND HEART RATES OF 330 BEATS/MIN.

SUBSTRATE COMBINATION	STROKE VOLUME ml/g	CORONARY FLOW ml/g/min	MYOCARDIAL OXYGEN CONSUMPTION µl O2/g/min	L.V. PRESSURE mmHg	L.V. MAX +VE dP/dt mmHg/sec	L.V. MAX -VE dP/dt mmHg/sec	RELAXATION TIME msec	END-DIASTOLIC PRESSURE mm Hg	HEART WORK mW/g	EFFICIENCY Joules/ml O_2
11,1 mM Glucose (3% Albumin)	1,7 ±0,1 (7)	203,6 ±5,0 (7)	2035 ±107 (7)	172,1 ±5,5 (6)	3573 ±87 (6)	2800 ±97 (6)	80,8 ±1,9 (6)	15,6 ±2,0 (6)	154,2 ±14,0 (7)	4,6 ±0,4 (7)
1 mM Palmitate (3% Albumin)	1,5 ±0,1 (8)	212,8 ±9,4 (8)	2221 ±153 (8)	155,9 ±3,5 (8)	3500 ±45 (8)	2610 ±108 (8)	84,8 ±2,0 (8)	16,5 ±0,7 (8)	141,6 ±15,6 (8)	4,0 ±0,4 (8)
10 mM Lactate	1,8 ±0,1 (7)	214,8 ±5,5 (7)	2340 ±75 (6)	151,2 ±4,3 (7)	3474 ±60 (7)	2571 ±111 (7)	87,0 ±2,3 (7)	15,4 ±1,4 (7)	161,3 ±8,8 (6)	4,2 ±0,3 (6)
10 mM Pyruvate 11,1 mM Glucose Insulin 2 U/L	1,9 ±0,1 (6)	206,7 ±10,5 (6)	2198 ±129 (6)	175,8 ±5,1 (6)	3907 ±48 (6)	2747 ±35 (6)	91,7 ±2,2 (6)	19,7 ±0,7 (6)	206,5 ±16,6 (6)	5,6 ±0,3 (6)
1 mM Palmitate (3% Albumin) 11,1 mM Glucose Insulin 2 U/L	2,0 ±0,1 (6)	253,3 ±8,3 (6)	2498 ±122 (6)	195,7 ±4,9 (6)	3653 ±27 (6)	2720 ±174 (6)	80,0 ±1,4 (6)	17,8 ±0,7 (6)	191,1 ±7,1 (6)	4,6 ±0,1 (6)
11,1 mM Glucose	1,9 ±0,1 (10)	211,7 ±6,0 (10)	2101 ±69 (8)	169,5 ±6,3 (8)	3500 ±45 (8)	2896 ±72 (8)	80,2 ±1,8 (8)	15,2 ±0,9 (10)	180,1 ±9,7 (10)	5,1 ±0,3 (8)
10 mM Lactate 11,1 mM Glucose Insulin 2 U/L	2,2 ±0,1 (7)	226,6 ±9,2 (7)	2424 ±76 (7)	172,4 ±2,2 (7)	3737 ±29 (7)	2983 ±67 (7)	80,4 ±0,9 (7)	15,4 ±1,0 (7)	232,2 ±12,1 (7)	5,8 ±0,3 (7)
11,1 mM Glucose Insulin 2 U/L	2,2 ±0,1 (7)	220,0 ±6,2 (7)	2410 ±80 (7)	177,2 ±4,0 (7)	3623 ±23 (7)	3074 ±80 (7)	81,4 ±1,1 (7)	14,7 ±1,0 (7)	225,6 ±7,1 (7)	5,6 ±0,2 (7)

Note that, in general, stroke volumes increase in parallel with increases in LV max -ve dP/dt values.

Values are Mean ± SEM (number of measurements).

relate to:

(iv) Effects of perfusate albumin on heart function.

(v) Effects of different substrate combinations on calculated myocardial efficiency.

(vi) Effect of the left ventricular catheter on heart function.

(i) Effects on left ventricular function of the addition of insulin and glucose to the primary substrates - lactate, pyruvate, glucose and palmitate.

The addition of insulin to glucose and glucose plus insulin to lactate or palmitate increased stroke volumes, calculated heart work, and rates of both coronary flow and myocardial oxygen consumption (Table 5.7). Peak left ventricular pressures and the maximum rates of left ventricular pressure rise (L.V max +ve dP/dt) and fall (L.V. max -ve dP/dt) also increased. Calculated myocardial efficiencies were improved when glucose plus insulin was added to hearts perfused with lactate and palmitate, but the addition of insulin to glucose did not improve calculated myocardial efficiencies.

The addition of glucose and insulin to lactate and palmitate-perfused hearts reduced left ventricular relaxation times,whereas relaxation times were similar in hearts perfused with either glucose or glucose plus insulin. End-diastolic pressures were unaffected by the addition of glucose plus insulin to the primary perfusion substrates, but this pressure was higher in hearts perfused with non-glucose (lactate, pyruvate, palmitate) than with glucose as substrate.

Thus,insulin is required in the perfusion fluid if peak myocardial mechanical function is to be achieved during perfusions with any

primary substrate. Its addition improves myocardial efficiency and increases parameters of both myocardial contractility and myocardial relaxation.

(ii) Comparison of left ventricular function in hearts perfused with either glucose, glucose plus insulin, or glucose plus pyruvate and insulin.

Figure 5.5 compares left ventricular function of hearts perfused with either glucose, glucose-insulin or glucose-pyruvate-insulin at different atrial filling pressures and heart rates. The striking finding was that,despite significantly greater end-diastolic pressures and greater rates of left ventricular pressure development, stroke volumes and calculated heart work were,at the higher atrial filling pressures, significantly lower in glucose-pyruvate-insulin than in glucose-insulin perfused hearts. Thus,the significantly smaller stroke volumes in glucose-pyruvate-insulin perfused hearts cannot be explained on the basis of their significantly higher rates of left ventricular pressure development or end-diastolic pressures, both of which would have been expected to increase stroke volumes.

One is therefore left to conclude that the significantly greater rates of left ventricular relaxation and the shorter relaxation times in the glucose-insulin perfused hearts must be the factor explaining their greater stroke volumes. This suggests that stroke volume in the isolated perfused working rat heart may be limited by factors occurring during diastole. Thus,the smaller stroke volumes in glucose-pyruvate-insulin perfused hearts would be partially explained by reduced diastolic filling due to their slower rates of myocardial relaxation. The significantly higher end-diastolic pressures measured in these hearts would also impair diastolic filling. This latter finding also indicates that myocardial compliance is altered by the nature of substrates supplied to the heart,

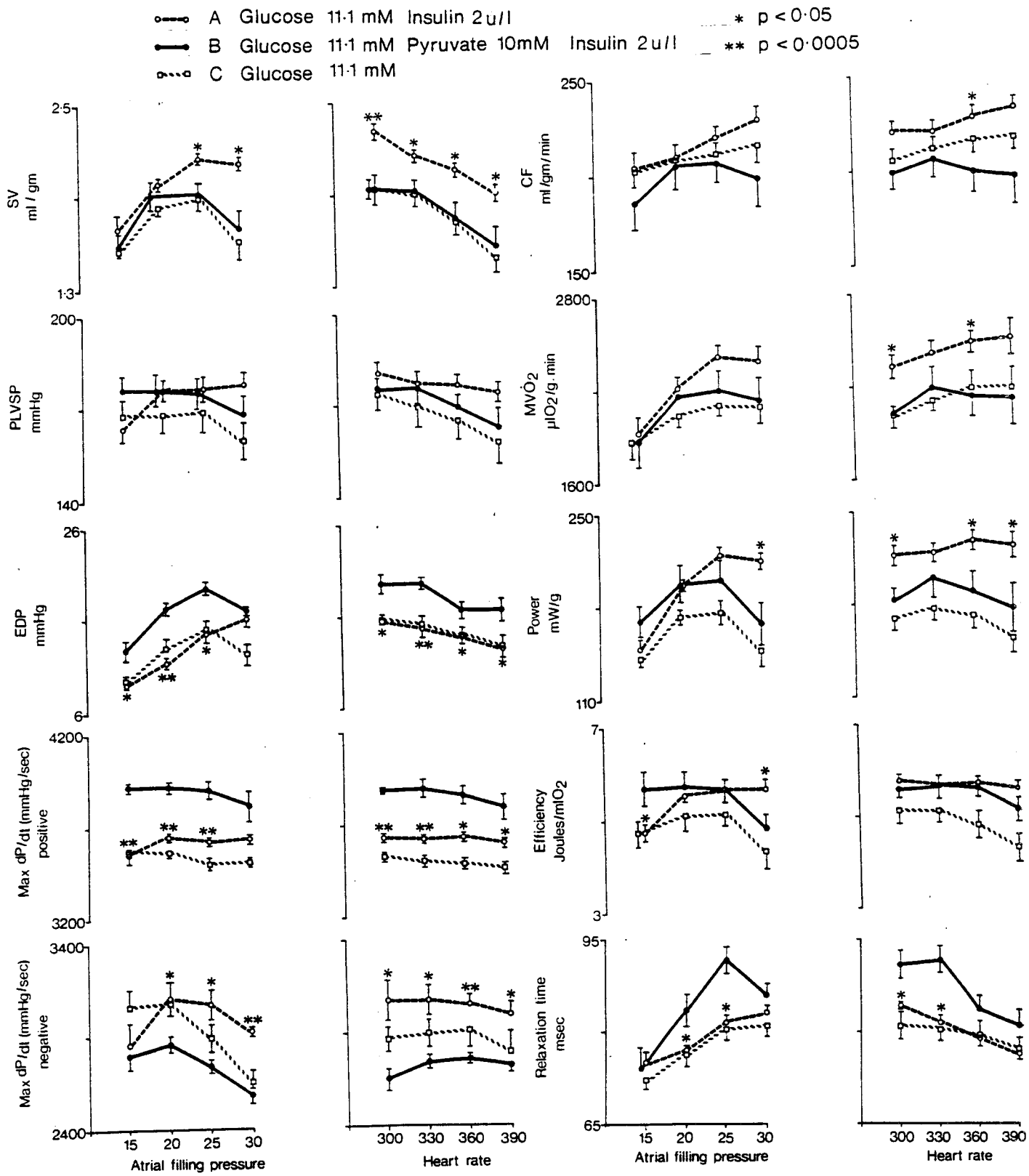

Figure 5.5

Comparison of left ventricular function of hearts perfused with glucose, glucose-insulin, and glucose - pyruvate - insulin under conditions of changing atrial filling pressures and heart rates.

Note higher L.V. max + ve dp/dt in hearts perfused with

glucose - pyruvate - insulin but lower stroke volumes and L.V. max - ve dp/dt.

Abbreviations:

CF	-	Coronary flow
$M\dot{V}O_2$	-	Myocardial oxygen consumption
SV	-	Stroke volume
PLVSP	-	Peak left ventricular systolic pressure
EDP	-	End diastolic pressure

Each point represents Mean ± SEM for at least 6 measurements.

and is reduced in non-glucose perfused hearts.

Peak left ventricular systolic pressures were not significantly different between glucose-insulin and glucose-pyruvate-insulin perfused hearts, whereas myocardial efficiencies, rates of coronary flow and myocardial oxygen consumption were greater in glucose-insulin perfused hearts. However, these latter differences achieved statistical significance only at isolated points.

(iii) Evidence that the perfusion conditions used in section 5.3B produced maximum heart function.

Figure 5.5 shows that left ventricular power outputs are highest in hearts perfused with glucose-insulin at atrial filling pressures of 25 cmH_2O and at heart rates of between 330 and 390 beats/min. Stroke volumes and power outputs did not increase further when atrial filling pressures were increased to 30 cmH_2O. From this it is concluded that the perfusion conditions used in those studies reported in section 5.3B did indeed elicit peak left ventricular function. Thus, the conditions most likely to demonstrate differences in heart function between trained and control animals have been established by these studies, and these conditions were used to study the effects of exercise training on myocardial function and metabolism (Chapter 6).

Another feature demonstrated in Figure 5.5 is that stroke volumes, peak left ventricular systolic pressures and end-diastolic pressures all fall as heart rates are increased at atrial filling pressures of 25 cmH_2O. These findings are identical to those reported in the open-chested anaesthetized dog model, by Ilebekk and his colleagues[456,457]. They reported that as heart rates were artificially increased by atrial pacing, stroke volumes and end-diastolic myocardial cord lengths fell. They therefore concluded that the pacing-induced reduction in stroke volume

could be entirely explained on the basis of reduced activation of the Frank-Starling mechanism because, as heart rate increases,diastolic filling is reduced and end-diastolic pressure and myocardial sarcomere length falls.

Although left ventricular end-diastolic fibre lengths were not measured in this study, the similarity between these findings and those of Illebekk and his colleagues suggest that in the isolated perfused rat heart model, as heart rate increases, mechanical function is limited by reduced activation of the Frank-Starling mechanism.

(iv) Effects of perfusate albumin on heart function.

An important difference between hearts perfused with fatty acids (palmitate or palmitate-glucose-insulin) and those perfused with the other substrates, is that in the former the fatty acid is carried in combination with albumin. It is not known how this albumin affects either left ventricular pressure changes or cardiac outputs and calculated heart work. Theoretically, if the albumin significantly increases perfusion fluid viscosity, it will increase the resistance to flow of both the fluid entering, and being ejected from the left ventricle. The results would be:
(a) Reduced diastolic left ventricular filling, and therefore reduced stroke volume if, in this model, stroke volume is principally limited by diastolic filling.
(b) An apparent reduction in heart work for a given amount of actual mechanical work performed by the heart. This undercalculation is inherent in the formula used for the calculation of heart work, because the formula is an approximation which does not take account of perfusion fluid viscosity[458]. Therefore, calculated heart work will, when compared to hearts perfused with control solutions,

be underestimated in hearts perfused with solutions of increased viscosity, because such hearts will, in ejecting the more viscous fluid, expend more energy than is calculated.

c) The addition of albumin reduces the perfusate ionized calcium concentration by up to 30-50% and could therefore influence left ventricular performance.

Table 5.7 and figure 5.6 show that, when compared to glucose-perfused hearts, hearts perfused with glucose-albumin have significantly lower stroke volumes, they apparently perform less work, and have impaired myocardial efficiency. These occur despite there being no significant differences in either rates of coronary flow or myocardial oxygen consumption, in left ventricular max +ve or max -ve dP/dts, in peak left ventricular pressures, in left ventricular relaxation times, or in left ventricular end-diastolic pressures. This suggests that the perfusate albumin concentration used, did not produce functionally-significant changes in the perfusate ionized calcium concentration.

Thus, the addition of albumin to the perfusate reduced stroke volumes, heart work and myocardial efficiencies, none of which changes could be explained on the basis of a deleterious effect of albumin on left ventricular contractility. One must therefore conclude that the apparently deleterious effect albumin exerts on myocardial function results from the increased viscosity of the albumin-containing fluid which either impairs left ventricular filling or increases resistance to flow in the aortic column.

Evidence that reduced atrial filling may not be the principal factor is the finding that, at the highest heart rates (390 beats/min), stroke volumes were not different between glucose and glucose-albumin perfused hearts (Appendix 2.1 and Figure 5.6), whereas at low heart rates, stroke volumes were significantly lower in glucose-albumin perfused hearts. Thus, when diastolic filling times were reduced by increasing heart rates, stroke volumes fell more in hearts perfused with glucose than in those perfused with glucose-albumin, suggesting that

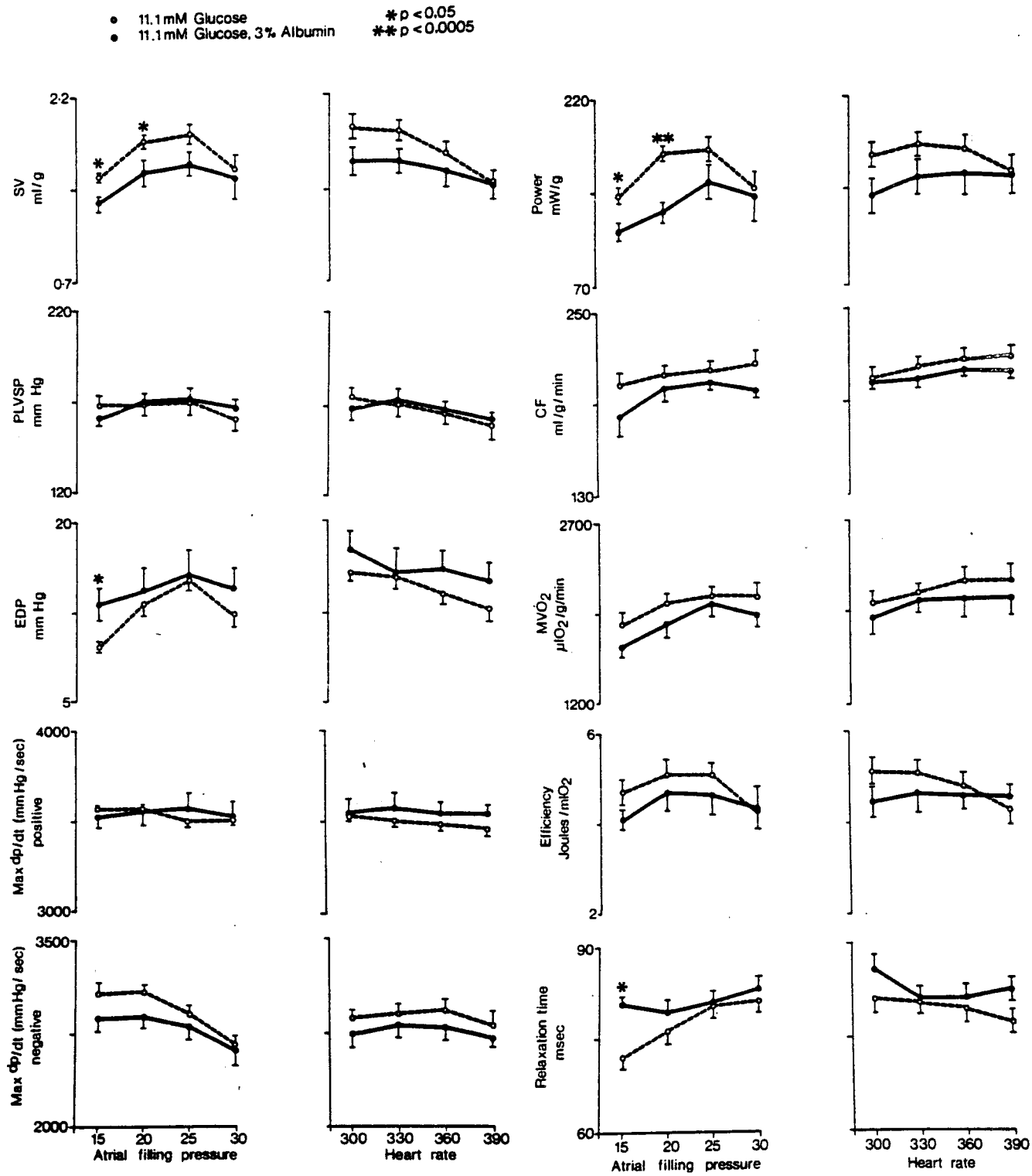

Figure 5·6

Comparison of left ventricular function of hearts perfused with either glucose or with glucose and albumin. Note that stroke volumes, calculated powers and efficiencies are lower in glucose-albumin hearts.

Each point on the graph represents Mean ± SEM for 6 measurements.

Abbreviations as for Figure 5·5

even at low heart rates, stroke volumes in glucose-albumin perfused hearts are limited principally by the increased resistance to aortic flow.

(iv) Effects of different substrate combinations on calculated myocardial efficiency.

For the same reason described above, namely that the formula for the calculation of heart work and therefore efficiency does not take account of perfusion fluid viscosity, myocardial efficiency will be underestimated in hearts perfused with solutions of increased viscosity. This would explain why calculated heart work and myocardial efficiency was lower in hearts perfused with glucose-albumin than with glucose, despite there being no other differences in parameters of left ventricular function, including rates of myocardial oxygen consumption. Therefore, at least part of the reason for the lower stroke volumes, heart work and reduced myocardial efficiencies of hearts perfused with palmitate or palmitate-glucose-insulin than with glucose or glucose-insulin (Table 5.7) will be due to the presence of albumin in the former solutions. For this reason, it is not possible to compare directly the overall heart function of hearts perfused either with or without albumin-containing solutions.

In this study, therefore, the lower calculated efficiencies of palmitate-perfused hearts cannot be ascribed only to the "oxygen-wastage" effect described by previous authors (section 5.2B). Similarly, the higher rates of myocardial oxygen consumption at similar levels of heart work that Neely, Whitner and Mochizuki[434] found in hearts perfused with palmitate-glucose when compared to glucose-perfused hearts, may also have been caused by the greater viscosity of the fatty acid solution, rather than by "oxygen-wastage". This criticism does not apply to those studies reporting an

"oxygen wastage" effect in which either the Langendorff, non-working heart[444, 445, 450] or in vivo models[446,447,449] were used.

On the other hand, in this study, calculated myocardial efficiency of palmitate-perfused hearts was significantly lower than that measured for hearts perfused with glucose-albumin at an atrial filling pressure of 25 cmH_2O when heart rates were either 360 or 390 beats/min, or at an atrial filling pressure of 30 cmH_2O with heart rate 330 beats/min (Table 5.8). As the perfusate viscosities would have been equal, this finding does indicate "oxygen wastage" by palmitate-perfused hearts. Furthermore, lactate-perfused hearts were also significantly less efficient than glucose-perfused hearts (Table 5.8),but myocardial efficiency was similar for hearts perfused with either glucose-insulin, glucose-lactate-insulin or glucose-pyruvate-insulin. Therefore,whatever the mechanism underlying the "oxygen wastage" effect of lactate, it is negated by the addition of glucose-insulin.

In summary, this study provides further evidence that there are substrate-related differences in myocardial efficiency, and that palmitate- and lactate-perfused hearts are less efficient than those perfused with either glucose-albumin or glucose respectively. By itself, the presence of albumin in the perfusion fluid reduces calculated myocardial efficiency but this is due to the increased perfusion fluid viscosity that it causes.

(vi) Effect of the left ventricular catheter on heart function.

It should be noted that under comparable perfusion conditions (heart rates 330 beats/min, atrial filling pressures 25 cmH_2O, aortic column height 120 cm), the presence of a left ventricular catheter caused an approximate 13% reduction in stroke volume (compare results from

TABLE 5.8

CALCULATED MYOCARDIAL EFFICIENCIES (Joules/ml O_2) OF HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS UNDER CONDITIONS OF CHANGING ATRIAL FILLING PRESSURES AND HEART RATES.

SUBSTRATE COMBINATION		HEART RATE - 330 BEATS/MIN ATRIAL FILLING PRESSURE (cm H_2O)				ATRIAL FILLING PRESSURE - 25 cm H_2O HEART RATE (BEATS/MIN)			
		15	20	25	30	300	330	360	390
A.	1 mM Palmitate (3% Albumin)	3,8 ±0,4 (8)	3,7 ±0,2 (8)	4,0 ±0,4 (8)	2,7 ±0,2 (8)	3,6 ±0,3 (8)	4,0 ±0,4 (8)	3,2 ±0,4 (8)	2,8 ±0,3 (8)
B.	11,1 mM Glucose (3% Albumin)	4,1 ±0,2 (7)	4,7^{+} ±0,4 (7)	4,6 ±0,4 (7)	4,3^{++} ±0,5 (7)	4,4 ±0,3 (7)	4,6 ±0,4 (7)	4,6^{++} ±0,3 (7)	4,5^{++} ±0,3 (7)
C.	11,1 mM Glucose	4,7 ±0,3 (9)	5,1** ±0,3 (8)	5,1 ±0,3 (8)	4,3 ±0,4 (8)	5,1 ±0,3 (8)	5,1 ±0,3 (8)	4,8* ±0,3 (8)	4,3** ±0,3 (8)
D.	10 mM Lactate	3,4 ±0,2 (6)	3,7 ±0,2 (6)	4,2 ±0,3 (6)	3,8 ±0,3 (6)	4,1 ±0,4 (6)	4,2 ±0,3 (6)	3,6 ±0,3 (6)	3,5 ±0,3 (6)
E.	10 mM Lactate 11,1 mM Glucose Insulin 2 U/Litre	4,9^{xx} ±0,3 (7)	5,5^{xx} ±0,4 (7)	5,8^{xx} ±0,3 (7)	5,0 ±0,6 (7)	5,7^{x} ±0,4 (7)	5,8^{xx} ±0,3 (7)	5,8^{xx} ±0,3 (7)	5,4^{xx} ±0,4 (7)
F.	10 mM Pyruvate 11,1 mM Glucose Insulin 2/Litre	5,7 ±0,4 (6)	5,7 ±0,3 (6)	5,6 ±0,3 (6)	4,8 ±0,3 (6)	5,6 ±0,2 (6)	5,6 ±0,3 (6)	5,6 ±0,2 (6)	5,1 ±0,3 (6)
G.	11,1 mM Glucose Insulin 2 U/Litre	4,7 ±0,2 (7)	5,5 ±0,1 (7)	5,6 ±0,2 (7)	5,6$^{\theta}$ ±0,2 (7)	5,7 ±0,1 (7)	5,6 ±0,2 (7)	5,7$^{\theta}$ ±0,1 (7)	5,5$^{\theta}$ ±0,2 (7)
H.	1 mM Palmitate 11,1 mM Glucose (3% Albumin) Insulin 2 U/Litre	4,3 ±0,2 (6)	4,5** ±0,1 (6)	4,6 ±0,1 (6)	4,0* ±0,5 (6)	4,6* ±0,2 (6)	4,6 ±0,1 (6)	4,3 ±0,1 (6)	4,0* ±0,2 (6)

Note that at the highest heart rates, glucose-albumin and glucose perfused hearts are more efficient than are hearts perfused with palmitate-albumin or lactate respectively, and that the addition of (glucose)-insulin to lactate, palmitate or glucose-perfused hearts increases myocardial efficiency.

+	$p < 0,05$	B vs A	++	$p < 0,02$	B vs A
*	$p < 0,05$	C vs D	**	$p < 0,005$	C vs D
x	$p < 0,05$	E vs D	xx	$p < 0,005$	E vs D
•	$p < 0,05$	H vs A	θ	$p < 0,02$	G vs C
			••	$p < 0,005$	H vs A

Values are Mean ± SEM (number of measurements).

Table 5.1 and 5.2 with Table 5.7). The possible reasons for this may be either that the intraventricular space occupied by the catheter impairs myocardial filling thereby reducing end-diastolic volume, or it may interfere with normal ejection, or alternatively the passage of the catheter through the mitral valve and the apex of the heart may cause valvular or myocardial damage.

In more detailed studies[458] not reported here, it was found that in the absence of a left ventricular catheter, stroke volume and heart work did not fall at the highest left atrial filling pressure (30 cmH_2O) as they did in these studies. Furthermore, total heart work at an atrial filling pressure of 25 cmH_2O was approximately 30% higher when hearts were perfused without a left ventricular catheter in situ.

Thus,it is clear that in this model, the presence of a left ventricular catheter significantly alters heart function, and this should be taken into account whenever such studies are performed.

Summary and implications of initial studies.

These initial studies had clearly defined the perfusion conditions necessary for maximum function in the isolated perfused working rat heart model. They had also provided suggestive evidence that stroke volume in this model is limited by phenomena occurring in diastole, because differences in rates of myocardial relaxation seemed to be the only explanation for the finding of substrate-induced differences in stroke volume. This possibility was in line with the work of Buckley, Sidky and Ogden[459] who concluded that,in the isolated dog heart, stroke volume is determined early in diastole by conditions which may be partly measured in terms of mechanical impedance during left ventricular filling.

Finally, an apparent relationship between the rates of myocardial relaxation and of myocardial glycolytic ATP production was suggested by the finding that hearts perfused with solutions allowing the highest glycolytic ATP production rates also had the highest rates of myocardial relaxation.

Although these observations did not at first seem to relate directly to the specific topic of this thesis, I considered them to be of sufficient interest to merit some additional work. Therefore, I chose to do 2 additional series of experiments to see whether I could provide further experimental evidence that diastolic phenomena limit heart function in this model, and that glycolytically-produced ATP plays a special role in myocardial relaxation.

Effects of isoproterenol infusion on left ventricular function and myocardial metabolism.

Experimental protocols.

To study further the factors limiting heart function in this model, the β-receptor agonist isoproterenol was added to a series of hearts working under the maximum steady-state conditions defined in the previous section. The rationale for studying myocardial function during β-stimulation was that such stimulation is known to prolong diastolic filling time

(i) by reducing the duration of systole[460-466] thereby increasing the time available for left ventricular filling,

(ii) by increasing the rapidity of myocardial relaxation[462-466], and

(iii) reducing the ventricular impendance to filling[459,467].

If the function of the isolated working heart was indeed limited by myocardial relaxation, then the addition of catecholamines (

"the guardians of diastole[463]") to perfused hearts must cause their function to increase. Furthermore, catecholamine should cause increased rates of glycolytic ATP production, if such ATP production is linked to myocardial relaxation.

The perfusion protocol used in this group of experiments was as follows: the heart was mounted in the conventional manner, and the left ventricular catheter introduced as described previously. After left atrial cannulation had been completed, a 15-minute control period of left atrial perfusion commenced, during which cardiac outputs and rates of myocardial oxygen consumption were measured, and aortic and left ventricular pressure traces taken at 5 and 15 minutes. Prior studies not reported here, had shown that under these perfusion conditions, concentrationsof $6{,}5 \times 10^{-7}$Misoproterenol produced a maximum increase in cardiac output and calculated heart work. Higher isoproterenol concentrations were usually arrhythmogenic and did not further increase heart function. This drug concentration also increased the rate of unpaced perfused hearts to approximately 380 beats/min. Therefore, in these studies, hearts were paced at rates of 390 beats/min both in the control and infusion periods, to ensure that after isoproterenol infusion true catecholamine-dependent changes in heart function could be separated from changes resulting from increased heart rates.

After 15 minutes' left atrial perfusion, isoproterenol was infused into the perfusion fluid via side tubing connected immediately proximal to the atrial bubble trap. The infusion was delivered by a constant infusion pump (Braun, Melsungen, West Germany) and the syringe and tubing leading to the perfusion apparatus were protected from light by a black plastic covering. The isoproterenol solution was prepared immediately prior to its infusion by diluting 500 µl isoproterenol

hydrochloride (Isuprel(R) - Winthrop Laboratories) into 49,5 ml distilled water for infusion at a rate of 0,2 ml/min. At coronary flow rates of between 25-30 ml/min, this infusion rate gave a final isoproterenol concentration in the coronary circulation of approximately 6,5 x 10^{-7}M.

As oxidized isoproterenol is known to have a cardiotoxic effect[468] the perfusion apparatus was modified so that it became a non-recirculating working heart system, from which the coronary effluent was allowed to escape. To maintain the volume of circulating fluid, fully oxygenated pre-warmed fluid was constantly fed from an additional reservoir directly into the main reservoir of the working heart system (Fig. 5.1).

The non-recirculating system was necessary for the following reasons: (i) It allowed a constant catecholamine infusion to be used. This is potentially important because a constant catecholamine infusion produces a different, apparently more controlled myocardial cyclic AMP response than does the addition of a catecholamine bolus to a non-recirculating system[469]. (ii) A drip-through system was necessary for the measurement of glycolytic rates by the ^{3}H-glucose method described in Appendix 1.C.

At time intervals of 5, 15 and 25 minutes after the commencement of the isoproterenol infusion, cardiac outputs and myocardial oxygen consumption rates were measured, and aortic and left ventricular pressure traces were taken. Forty minutes after left atrial perfusion had commenced hearts were clamped in Wollenberger tongs and prepared for later biochemical analysis as previously described.

In a separate series of experiments hearts were perfused under identical conditions, but without a left ventricular catheter in situ, for measurements of glycolytic ATP production rates. ^{3}H-glucose (New England Nuclear, Boston, Massachussets) was added to the perfusate, as described

in Appendix 1.B, and coronary flow was sampled every 5 minutes. The experiments were terminated after 40 minutes' perfusion by clamping the hearts in pre-cooled Wollenberger tongs.

Samples of coronary flow were analyzed for their $^{3}H_2O$ glucose content according to the methods described in Appendix 1.C, and the hearts extracted and assayed for their ATP, PCr, glycogen, citrate and cyclic AMP levels, according to the methods described in Appendix 1.D. Rates of glycolytic ATP production were then calculated according to the equations described in Appendix 1.F.

Results:

Figure 5.7 shows that within 5 minutes of isoproterenol infusion, stroke volumes, end-diastolic pressures, peak left ventricular systolic pressures, rates of left ventricular relaxation, power outputs and rates of coronary flow and myocardial oxygen consumption had all increased, relaxation times and aortic ejection times had shortened and the maximum rates of left ventricular pressure development had remained unchanged. Thus, as hoped, catecholamine infusion had increased both stroke volume and the maximum rate of myocardial relaxation. Furthermore, as shown in Table 5.9, isoproterenol infusion increased rates of myocardial glycolytic ATP production.

It should be noted that the rates of glycolytic ATP production after 15 minutes steady-state left atrial perfusion were, when measured by the ^{3}H-glucose method, not different from the values calculated from substrate uptake data during 70 minutes steady-state perfusions (11,59 $\pm$ 0,49 vs 12,86 $\pm$ 1,02 (Table 5.6) µmol ATP/g/min). This indicates

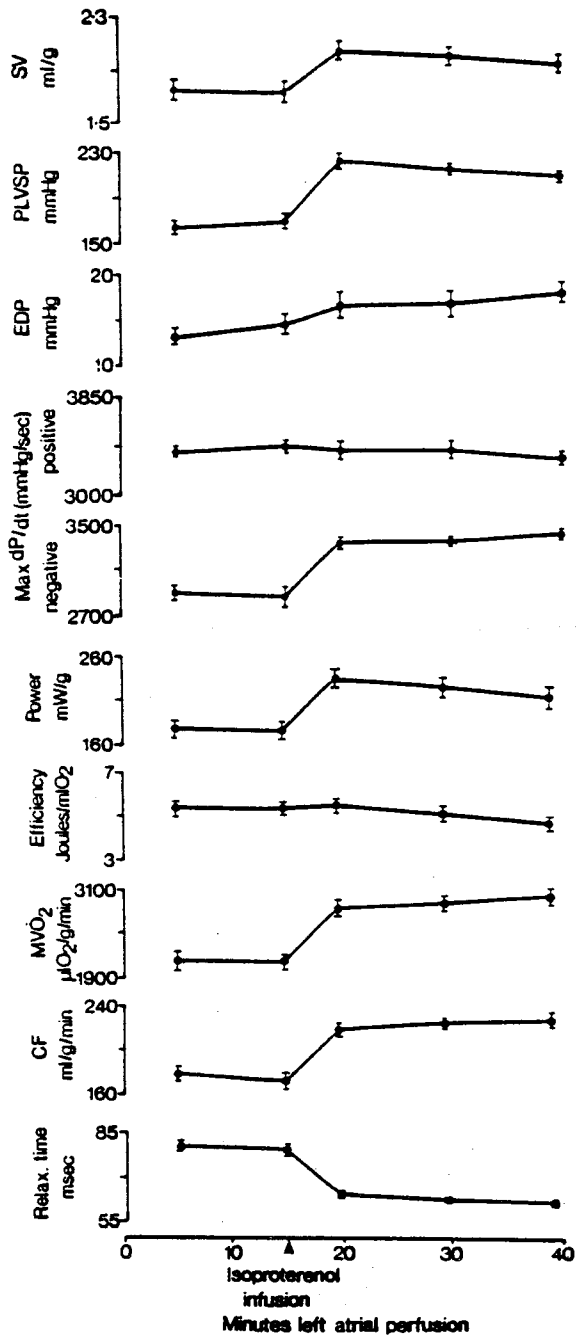

Figure 5·7

Effects of isoproterenol infusion on left ventricular function of isolated perfused working hearts. Note that most parameters of left ventricular function increase after isoproterenol infusion. Each point on the graph represents data from 8 experiments.
Abbreviations as for Figure 5·5

TABLE 5.9

RATES OF GLYCOLYSIS, GLYCOGENOLYSIS AND GLYCOLYTIC ATP PRODUCTION IN WORKING HEARTS PERFUSED UNDER STEADY-STATE CONDITIONS FOR 15 MINUTES WITHOUT, AND FOR AN ADDITIONAL 25 MINUTES WITH A CONSTANT $6,5 \times 10^{-7}$M ISOPROTERENOL INFUSION.

TIME (min)		GLYCOLYTIC * RATE µmol glucose equiv/g/min	GLYCOGEN ** UTILIZATION µmol glucose equiv/g/min	GLYCOLYTIC ATP PRODUCTION µmol/g/min
5		4,93 ±0,27		10,61 ±0,54
10		5,29 ±0,27	0,25	11,32 ±0,54
15		5,42 ±0,24		11,59 ±0,49
20	ISOPROTERENOL INFUSION	6,21 ±0,26		13,28 ±0,53
25		6,94 ±0,17		14,74 ±0,38
30		6,78 ±0,14	0,29 ±0,02	14,42 ±0,32
35		7,06 ±0,12		14,98 ±0,30
40		7,00 ±0,18		14,86 ±0,40

Note that glycolytic ATP production rates increase during isoproterenol infusion.

Data are mean ± SEM for 8 experiments.

* Glycolytic rates were measured by the ^{3}H-glucose method.

** Rates of glycogen utilization were calculated according to methods described in the text.

that calculation of glycolytic ATP production rates from substate uptake data (Table 5.6) gives results which compare favourably with directly measured values using the ^{3}H-glucose method.

The effect of isoproterenol infusion on tissue high energy phosphate and citrate levels is shown in table 5.5.

Effects of deoxyglucose on left ventricular function.

Experimental protocol.

To study further the relationship between myocardial glycolysis and left ventricular relaxation, the effects of glycolytic inhibition with deoxyglucose[470] (2-deoxy-D-glucose - Sigma Chemical Company) were studied in a re-circulating working heart system using the same steady state perfusion protocol described in section 5.3B but with a left ventricular catheter in situ. The perfusion substrates were either 10 mM L(+)lactate or 10 mM L(+)lactate plus 10 mM deoxyglucose. A separate series of hearts were perfused with 10 mM L(+)lactate plus 20 mM deoxyglucose, but, as the results of these experiments were not different from those measured in hearts perfused with the lower deoxyglucose concentration, these results are not presented.

At 5, 20, 35, 50 and 70 minutes after left atrial perfusion had commenced, cardiac outputs were measured and aortic and left ventricular pressure traces were taken. In addition, rates of myocardial oxygen consumption were measured at 35 and 70 minutes.

Results:

The results of these experiments are presented graphically in Figure 5.8. They show that 10 mM deoxyglucose had specific effects on both myocardial relaxation and on coronary flow rates. Thus, hearts perfused with lactate and deoxyglucose had significantly slower rates of

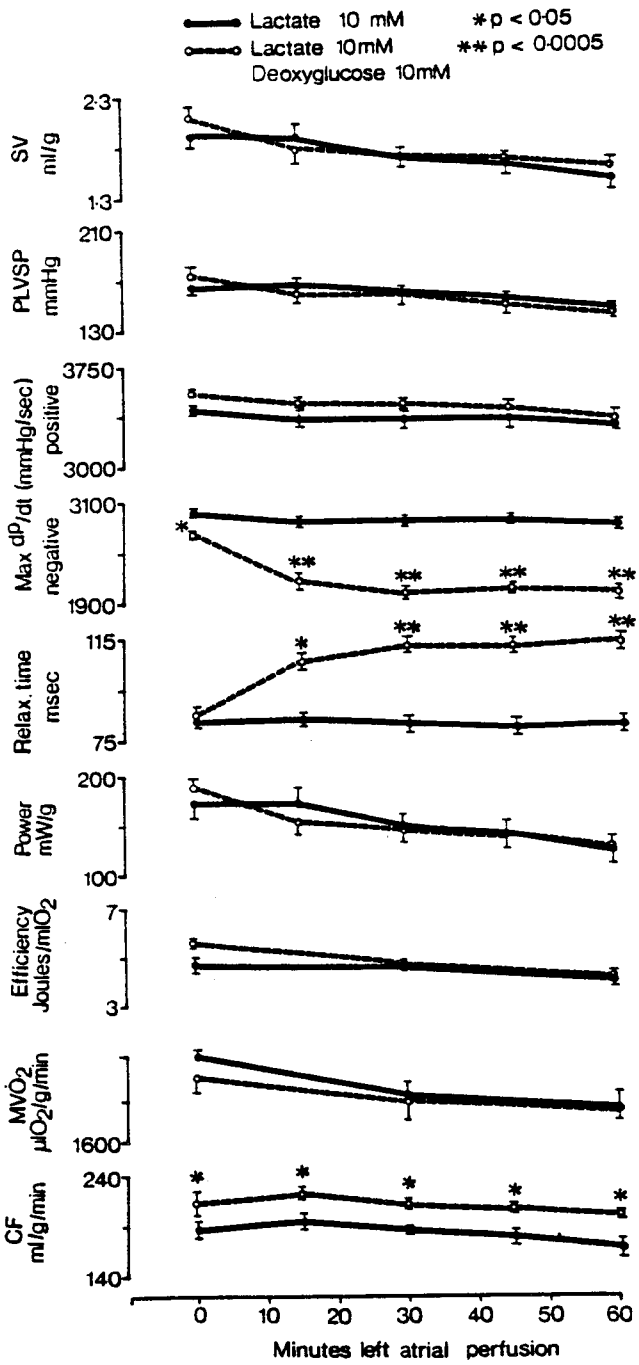

Figure 5·8

Comparison of left ventricular function of hearts perfused with either lactate or lactate plus deoxyglucose.
Note that deoxyglucose has a specific effect on myocardial relaxation, significantly reduces LV max-$^{dP}/_{dt}$ values and prolonging relaxation time. Deoxyglucose also caused significantly greater coronary flow rates.
Each point represents data from 5 experiments.
Abbreviations as for Figure 5·5

left ventricular relaxation, significantly longer relaxation times, and increased coronary flow rates.

These studies are therefore compatible with the hypothesis that glycolytically-produced ATP may play a special role in the processes of myocardial relaxation.

5.4 DISCUSSION AND CONCLUSIONS.

The principal conclusions from these studies can be summarized as follows:

(i) Peak heart function in the isolated perfused working rat heart is achieved at an atrial filling pressure of 25 cmH_2O, at a heart rate of 330 beats/min and with an aortic column height of 120 cms.

Additional studies not reported here[458], in which heart function was more accurately measured with the aid of a computer, have confirmed this conclusion.

(ii) Peak heart function in the isolated perfused working rat heart is <u>not</u> normally limited by the perfusate oxygen content.

It has been suggested that oxygenation of the isolated perfused working rat heart model is inadequate and may be a factor limiting its maximum function.[471] Three lines of evidence provided by my studies refute this suggestion. First, the highest myocardial oxygen consumption rates and the greatest levels of heart work were measured in hearts perfused with glucose-insulin to which isoproterenol had been added (Figure 5.7). Thus, it is extremely unlikely

that the mechanical performance of hearts perfused with the other substrate combinations, and which did not achieve such high rates of myocardial oxygen consumption or heart work, could have been limited by low perfusate oxygen tensions. Second, coronary flow rates were also highest in glucose-insulin perfused hearts to which isoproterenol had been added (Figure 5.7). Thus "coronary flow reserve" must have been present in hearts perfused with all the other substrate combinations. Had hypoxia been present, one would have expected coronary flow rates to have been maximal even before isoproterenol was infused. Third, data presented in Table 5.4 shows that myocardial ATP levels of hearts perfused with glucose-insulin for 70 minutes were the highest of all substrate groups and were equal to values measured in hearts perfused for 15 minutes in the Langendorff, non-working model. Had the high work loads achieved by glucose-insulin perfused hearts caused them to become hypoxic, one would have expected tissue ATP levels to have been reduced.

These arguments do not, of course, exclude the possibility that, after isoproterenol infusion, oxygen availability limited myocardial function in this model.

(iii) Peak heart function in the isolated perfused working rat heart is achieved by hearts perfused with either glucose-insulin or glucose-lactate-insulin.

This finding was quite contrary to what had been expected from the studies of Neely, Whitmer and Mochizuki[434], and Kobayashi and Neely[440]. These authors[434] reported that working hearts perfused only with glucose appeared to be metabolically

"run down" as indicated by low tissue levels of acetyl-CoA, citrate and isocitrate, and low NADH/NAD ratios. As myocardial oxaloacetate levels were high, they suggested that flux through the Krebs cycle was limited by the availability of acetyl-CoA. The phosphate potential of glucose-perfused hearts was also low, indicating a limitation of NADH for oxidative metabolism. Thus, they concluded that in hearts perfused only with glucose or with glucose and insulin, glycolysis cannot be accelerated sufficiently to provide adequate carbon substrate for energy metabolism. In contrast, the energy production of hearts perfused with palmitate, with or without glucose, did not appear to be substrate-limited as the mitochondrial NADH/NAD ratio and tissue levels of both acetyl-CoA and citric acid cycle intermediates were all high.

Further studies by Kobayashi and Neely[440] have compared the metabolism of lactate, glucose and pyruvate by the perfused rat heart. They showed that, whereas at increasing levels of heart work, substrate oxidation rates did not further increase when hearts were perfused with either glucose or lactate at concentrations above 15 mM or 10 mM respectively, hearts perfused with 10 mM pyruvate continued to increase pyruvate oxidation rates even at the highest work loads. Thus, at the highest levels of heart work, only pyruvate-perfused hearts were able to cover all their oxidative ATP requirements by the oxidation of exogenous substrate. At high workloads, oxidation of endogenous energy sources was required to make up the shortfall in ATP production caused by limitations of lactate and glucose oxidation rates. Therefore, from the metabolic data provided by these studies,

it would have seemed logical to assume that of the 4 primary substrates (glucose, lactate, pyruvate, palmitate), hearts perfused with pyruvate or palmitate would have reached the highest levels of heart work.

This study refutes that expectation, because although maximum rates of left ventricular pressure development were highest in pyruvate-glucose-insulin perfused hearts (Table 5.7), such hearts had stroke volumes and calculated power outputs which were amongst the lowest of all the groups studied.

Table 5.10 has been drawn up in an attempt to explain this apparent discrepancy on the basis of the relevant data collected in these studies. It lists results from hearts perfused with the different substrate combinations, in order of increasing rates of glycolytic ATP production. It will be seen that whereas stroke volumes and maximum rates of left ventricular relaxation increase, in order of increasing rates of glycolytic ATP production, maximum rates of left ventricular pressure development and myocardial citrate levels show no such trend.

Thus, one might propose that stroke volume in the isolated perfused working rat heart correlates best with indices of myocardial relaxation, which in turn may be related to rates of glycolytic ATP production.

(iv) Evidence that glycolytically-produced ATP plays a special role in myocardial relaxation.

Table 5.10 shows that stroke volumes and rates of left ventricular relaxation increase in parallel with increasing

TABLE 5.10

COMPARISON OF RATES OF GLYCOLYTIC ATP PRODUCTION, STROKE VOLUMES, LV MAX -VE AND MAX +VE dP/dt AND MYOCARDIAL CITRATE LEVELS IN HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS

SUBSTRATE COMBINATION	GLYCOLYTIC ATP PRODUCTION µmol/g/min	STROKE VOLUMES ml/g	LV MAX -VE dP/dt mmHg/sec	LV MAX +VE dP/dt mmHg/sec	MYOCARDIAL CITRATE µmol/g
1 mM Palmitate (3% Albumin)	0,37 ±0,04 (7)	1,50 ±0,14 (8)	2610 ±108 (8)	3500 ±45 (8)	0,57 ±0,05 (13)
10 mM Lactate	0,48 ±0,03 (9)	1,80 ±0,07 (7)	2571 ±111 (7)	3474 ±60 (7)	0,36 ±0,03 (15)
10 mM Pyruvate 11,1 mM Glucose Insulin 2 U/L	3,58 ±0,42 (6)	1,93 ±0,07 (6)	2747 ±35 (6)	3907 ±48 (6)	1,32 ±0,06 (11)
1 mM Palmitate (3% Albumin) 11,1 mM Glucose Insulin 2 U/L	6,12 ±1,07 (8)	2,00 ±0,08 (6)	2720 ±174 (6)	3653 ±27 (6)	0,64 ±0,05 (14)
11,1 mM Glucose	9,52 ±1,31 (7)	1,90 ±0,08 (10)	2896 ±72 (10)	3504 ±31 (10)	0,19 ±0,04 (16)
10 mM Lactate 11,1 mM Glucose Insulin 2 U/L	9,38 ±1,25 (6)	2,20 ±0,09 (7)	2983 ±67 (7)	3737 ±29 (7)	0,64 ±0,06 (12)
11,1 mM Glucose Insulin 2 U/L	12,86 ±1,02 (7)	2,16 ±0,04 (7)	3074 ±80 (7)	3623 ±23 (7)	0,28 ±0,03 (13)

Note that stroke volumes and rates of myocardial relaxation increase with increasing rates of glycolytic ATP production.

Values are mean ± SEM (number of measurements).

rates of glycolytic ATP production. Other data are in accord with these observations. Thus, isoproterenol infusion increased stroke volumes and rates of both myocardial relaxation (Fig. 5.7) and glycolytic ATP production (Table 5.9). Furthermore, when data from all these experiments were included, a significant correlation was found between the mean rates of glycolytic ATP production and the mean maximum rates of left ventricular relaxation (L.V. max -ve dP/dt)(Fig. 5.9). In addition, the metabolic inhibitor deoxyglucose, which is believed to exert a truly-specific metabolic inhibitory effect only on glycolysis through its action at the level of glucose phosphate isomerase[470], had a specific effect only on myocardial relaxation (Fig. 5.7). That myocardial oxygen consumption rates were unaltered by deoxyglucose addition indicates that respiratory ATP production rates were unaltered by this inhibitor.

The finding that even in the presence of 10 or 20 mM deoxyglucose, lactate-perfused hearts were still able to relax (i.e. that they did not develop myocardial contracture) indicates either that total glycolytic inhibition was not achieved, or that glycolytically-produced ATP is not the sole source of the energy required for myocardial relaxation. This latter interpretation is in accord with the data in Figure 5.9, which predicts that when glycolysis is totally-inhibited, L.V. max -ve dP/dt is approximately 2 550 mmHg/sec. The tentative conclusion from this work would therefore be that although respiratory ATP is able to supply ATP for myocardial relaxation, translocation of that ATP from the mitochondria to the sarcoplasmic reticulum occurs too slowly for there to be maximum

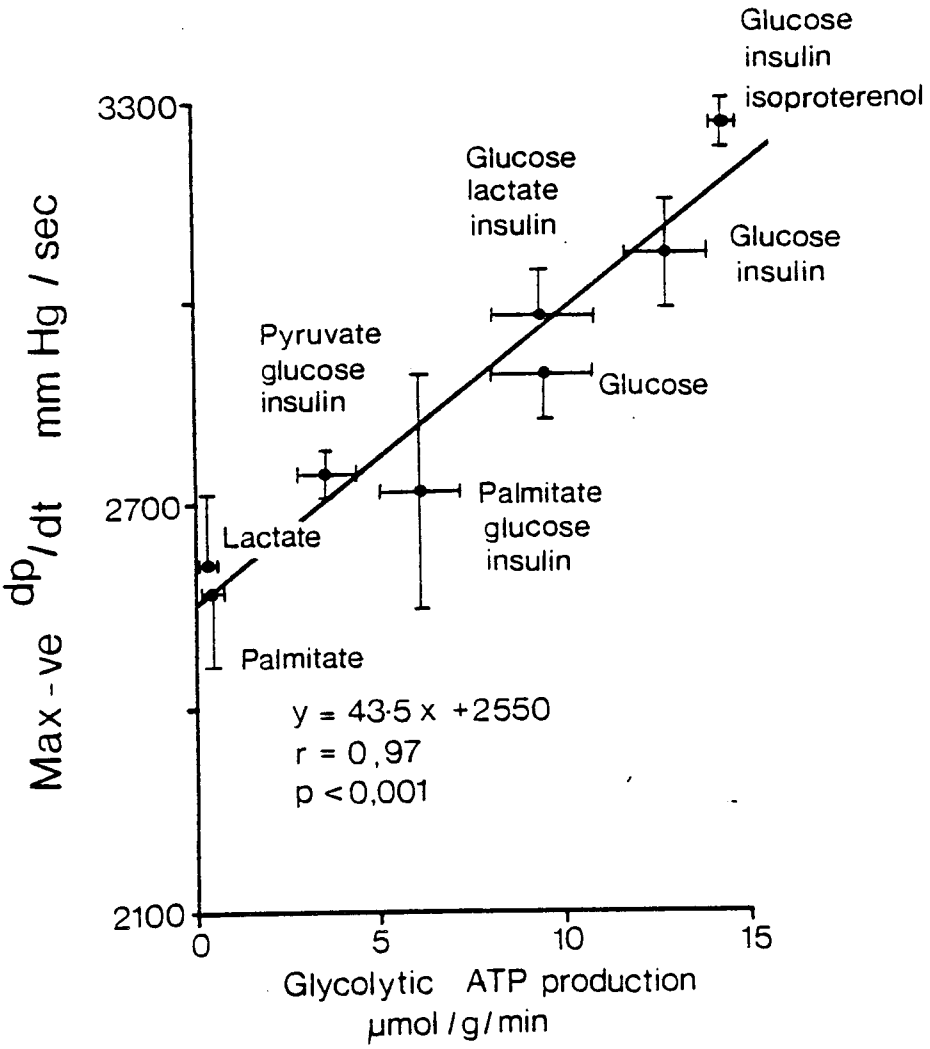

Figure 5·9

Graph showing significant correlation between the mean rates of myocardial glycolytic ATP production and the mean maximum rates of left ventricular relaxation (max-ve dp/dt) for hearts perfused with 8 different substrate combinations.

rates of left ventricular relaxation.

The hypothesis that glycolytically-produced ATP may play a special role in myocardial relaxation is consistent with the recently proposed concept that this ATP pool may serve a special role in myocardial metabolism. Thus, investigations of the effect of glucose on action potential duration have led to the postulate that glycolytically-produced ATP is used preferentially in the energy requiring membrane functions of cardiac muscle[472]. Furthermore, biochemical studies have established that glycogenolytic enzymes are presented in myocardial sarcoplasmic reticulum[473], and that creatine kinase is found in the myocardial endoplasmic reticulum fraction[474], a fraction which also probably represents sarcoplasmic reticulum.

Evidence that such enzyme localization may play a special role in myocardial relaxation comes from a number of recent studies. Anderson and Morris[475] showed that in the isolated rat right ventricular preparation, glucose prevented the rise in resting tension that occurred during anoxic perfusions with either a glucose-free medium or with one containing only 2-deoxyglucose. As developed tensions and tissue high energy phosphate levels were not different between groups of hearts perfused with the different substrates, these authors concluded that substrate-stimulated glycolysis must have provided sufficient ATP to maintain the required ATP level in a special ATP pool that is used specifically to support calcium uptake by the sarcoplasmic reticulum. In the words of these workers, these results indicated "a preferential utilization of glycolytically

produced ATP by the sarcoplasmic reticulum in the process of calcium uptake".

In isolated, isovolumically contracting rat hearts, Apstein, Deckelbaum, Hagopian and Hood[476] reported that total glycolytic blockade with iodoacetate reduced the time required for the development of irreversible myocardial contracture during acute global ischaemia from 30 minutes to 30 seconds. In as yet unpublished data that follow from their observation that myocardial contracture in isolated hearts exposed to low perfusion pressures is prevented by the addition of glucose to the perfusion medium[477], Bricknell, Daries and Opie[478] have shown that myocardial contracture in the same model occurs concurrent with the onset of glycolytic inhibition produced by a variety of inhibitors. In the final relevant study, Paul, Bauer and Pease[479] have established that glycolysis provides the energy required for cellular Na^+-K^+ transport in vascular smooth muscle.

If the above is indeed true and if, as argued in the previous section, the rate of myocardial relaxation limits stroke volume in the isolated perfused rat heart model, then the effect of a substrate on heart function in this model can be predicted from its effect on glycolysis. Thus, in combination with glucose-insulin, pyruvate and palmitate are inferior myocardial substrates than is lactate, because they are stronger glycolytic inhibitors (Table 5.10). Lactate on the other hand causes very little glycolytic inhibition (compare glycolytic rates for glucose-insulin and glucose-lactate-insulin perfused hearts - Table 5.10). This could explain why, as recently shown, lactate

is the preferred myocardial fuel[480], particularly during high-intensity exercise[481].

(v) Substrate-induced differences in myocardial efficiencies.

These studies confirm that myocardial efficiencies are different between substrate groups, and are reduced in hearts perfused in the absence of glucose-insulin.

Thus, at the highest heart rates and atrial filling pressures, lactate- and palmitate-perfused hearts were found to be less efficient than were hearts perfused with glucose and glucose-albumin respectively (Table 5.8). These findings are in general agreement with the published literature. A novel finding, however, was that the addition of insulin-(glucose) to glucose, lactate and palmitate perfused hearts increased their myocardial efficiencies.

It should be noted, however, that the equations[457] used for the calculation of the power output of the heart in these studies have subsequently been shown by us[458] to underestimate the actual total power output of hearts perfused in the presence of a left ventricular catheter by about 20% and by about 25% in hearts perfused in the absence of such a catheter. Thus myocardial efficiency, because it is calculated as power output divided by oxygen consumption, will also be underestimated by an equivalent amount.

The explanation for this is that the original equations developed by Kannengiesser, Opie and van der Werff[457] were based on an approximation which considers that the heart works as a continuous

flow pump. This was a necessary approximation because, until recently, it has been possible to measure only mean pressures and mean flow rates in this model.

However, a fundamental characteristic of the cardiovascular system is its pulsatility, because the heart works intermittently, not continuously. This pulsatile nature of contraction requires that with each beat, the isolated heart delivers additional kinetic power to accelerate the perfusate through the perfusion apparatus. To quantify this kinetic power component requires that pressures and flows be measured instantaneously by a minicomputer. When this was done[458], it was found that this kinetic component of the power output of the heart may constitute as much as 25% of its total power output.

Although it is unlikely that this kinetic component of the power output of the heart will be greatly different between groups of hearts perfused with the different substrate combinations, for a more accurate determination of the effects of different substrate combinations on myocardial efficiency, additional studies will need to use minicomputer techniques similar to those described in Chapter 6.

(vi) Relevance of these preliminary findings to studies of the myocardial adaptations to exercise training in the same model (Chapter 6).

These studies have shown that isolated hearts achieve maximum levels of cardiac performance when perfused at an atrial

filling pressure of 25 cmH_2O, at heart rates of 330 beats/min and with an aortic column height of 120 cm. Of all the substrate combinations tested, the highest levels of cardiac performance were reached by hearts perfused with either 11,1 mM D(+) glucose plus insulin at 2 units/litre or with 11,1 mM D(+) glucose and 10 mM L(+) lactate plus insulin at 2 units/litre. The addition of $6,5 \times 10^{-7}$M isoproterenol further increased performance of hearts perfused with the optimum glucose-insulin solution. The most likely explanation for the effects of the different substrate combinations on cardiac performance was their different effects on the rates of myocardial relaxation resulting, it is postulated, from the different degree to which each substrate combination inhibited myocardial glycolysis.

On the basis of these results, it seemed logical that hearts from trained and control rats should be perfused at the highest workload to determine whether differences in myocardial performance could be explained on the basis of alterations in the rates of both myocardial relaxation and of glycolytic ATP production. Furthermore, in view of the controversy surrounding the effects of exercise training on the myocardial contractile protein ATP hydrolysing activities (section 2.3C) it seemed desirable that further in-depth studies of that topic should be undertaken.

CHAPTER 6

MYOCARDIAL ADAPTATIONS TO EXERCISE TRAINING: PART 2.

MYOCARDIAL FUNCTIONAL AND METABOLIC ADAPTATIONS TO RUNNING TRAINING STUDIED IN THE ISOLATED PERFUSED WORKING RAT HEART MODEL.

6.1 INTRODUCTION

With the information gained from the studies reported in the previous chapter, it was possible to study, in the isolated rat heart model, the effects of exercise training on myocardial function and metabolism under those perfusion conditions that elicit maximum cardiac performance.

As reviewed in section 2.3D, Scheuer and his colleagues have used this model in their very extensive studies of the effects of exercise training, in particular swimming-training, on myocardial mechanical performance. Their studies have shown that hearts from male rats trained with a moderately strenuous swimming programme exhibit superior mechanical function as shown by a number of criteria: increased cardiac outputs[209, 251, 260-262], increased stroke work and maximum powers[261, 262], more rapid rates of circumferential fibre shortening from equivalent end-diastolic volumes[261, 262], more rapid rates of left ventricular relaxation[209, 251, 261, 262] and left ventricular pressure development[252, 260] associated with increased rates of both coronary flow and myocardial oxygen consumption[209, 260, 261].

Biochemical correlates for this superior heart function appear to be higher actomyosin and myosin ATPase activities associated with alterations in the sulphydryl group of myosin (section 2.3B). A more rapid rate of calcium uptake by isolated sarcoplasmic reticulum would explain the more rapid rates of myocardial relaxation in hearts from swimming-trained rats[199]. Swimming-trained hearts also had increased mitochondrial protein contents, but mitochondrial function expressed relative to tissue weight was not different between hearts from trained and control animals[182].

In contrast to these detailed studies on the effects of swimming training on the heart, there is only one similar study of the effects of treadmill running training. Schaible and Scheuer[262] compared myocardial function of isolated perfused hearts from rats trained with either swimming or running. They reported that although certain quantitative and qualitative differences were present, the myocardial functional adaptations to both training protocols were similar. Thus cardiac outputs, stroke work, maximum powers, ejection fractions and maximum velocities and extents of circumferential fibre shortening were, when compared to values in control hearts, all significantly higher in hearts from rats trained with either swimming or running. However, although the maximum rates of left ventricular relaxation (L.V. max -ve dP/dt) were higher in hearts from swimming-trained than from control rats, there was no difference when values from running-trained and control rats were compared. Thus,swimming training may have a specific effect on myocardial relaxation which is not produced by running training. This would be especially relevant if, as suggested by my studies in the previous chapter, rates of myocardial relaxation limit heart function under maximum conditions. It should also be noted that,in contrast to their previous reports,neither the maximum rates of left ventricular pressure development, nor peak left ventricular systolic pressures, nor coronary flow rates were significantly higher in hearts from swimming-trained than from control rats.

In this latter study, Schaible and Scheuer[262] did not measure either actomyosin or myosin ATPase activities in their running-trained rat hearts, but in a more recent paper, Penpargkul, Malhotra, Schaible and Scheuer[201] reported that there were only small increases in actomyosin ATPase activities in running-trained rats. Thus,the possibility exists that the superior myocardial function measured in hearts from running-trained

rats resulted from adaptations other than increased actomyosin or myosin ATPase activities. Furthermore, this finding casts doubt on the functional relevance of the increased actomyosin and myosin ATPase activities measured in hearts of swimming-trained animals.

In the studies reported in this chapter, I have extended these observations of the effects of running-training on myocardial function and metabolism. In particular, in collaboration with Ms. Thérèse Resink and Professor W. Gevers of the Department of Medical Biochemistry, University of Cape Town, the effects of running training on cardiac performance and myocardial actomyosin and myosin ATPase activities, troponin-1 and myosin P light chain phosphorylation have been studied in detail. Special attention has been paid to the role of calcium in mediating the effects of running training on the heart.

6.2 EXPERIMENTAL METHODOLOGY.

A. The rat exercise training programme.

This is described in Appendix 1.A.

B. Perfusion apparatus and perfusion techniques.

The perfusion apparatus and perfusion techniques used in these experiments have been described in detail in the previous chapter.

C. Perfusion fluids.

The perfusion fluid used in these experiments was the Krebs-Henseleit buffer described in Appendix 1.B. Two groups of hearts studied in those experiments described in section 6.5 were perfused with Krebs-Henseleit buffer at calcium concentrations of either 1,6 or 3,6 mM. In these experiments, the perfusate magnesium concentration was altered in parallel to the change in calcium concentration so that the perfusate Ca^{++}/Mg^{++} ratio remained constant.

D. Perfusion fluid analyses, tissue biochemical analyses, calculation and expression of results, and statistical methods.

These are described in Appendices 1.C, 1.D and 1.F.

E. Experimental protocols.

There were 3 different experimental protocols.

1) Seventy-minute, maximum steady state perfusions (section 6.3) in which overall mechanical performance and metabolism of hearts from trained and control animals were compared during perfusions with 6 different substrate combinations.

2) Experiments in which the left ventricular function of trained and control hearts was compared under conditions of changing atrial filling pressures and heart rates with 2 different substrate combinations (section 6.4).

3) Experiments in which left ventricular function, metabolism, and contractile protein ATP hydrolysing activities and phosphorylation levels were compared in trained and control hearts during maximum steady state perfusions with varying periods of isoproterenol infusion (section 6.5).

6.3 MECHANICAL PERFORMANCE AND METABOLISM OF HEARTS FROM TRAINED AND CONTROL RATS STUDIED UNDER STEADY STATE PERFUSION CONDITIONS.

Experimental protocol.

Prior to anaesthesia, all rats were weighed. In one series of 25 trained and 22 untrained rats, resting heart rates were measured on a Beckman polygraph (Beckman Instruments, Fullerton, California) with silver needles inserted subcutaneously, whilst the rats were lightly anaesthetized immediately prior to thoracotomy for removal of their hearts.

The hearts were mounted in the conventional manner and left atrial perfusion commenced after a 15-minute Langendorff pre-perfusion period. The experimental conditions used during working heart perfusion were those shown in the previous chapter to produce maximum cardiac performance (left atrial pressure 25 cmH_2O, heart rates 330 beats/min, aortic column height

120 cm). One series of hearts was also paced at 390 beats/min. Six different substrate combinations were studied, and these were:

11,1 mM D(+) glucose

11,1 mM D(+) glucose plus insulin at 2 u/litre

10 mM L(+) lactate

10 mM L(+) lactate and 11,1 mM D(+) glucose plus insulin at 2 u/litre

1 mM palmitate bound to 3% albumin

1 mM palmitate bound to 3% albumin and 11,1 mM D(+) glucose plus insulin at 2 u/litre.

Following an initial 5 minute working heart equilibration period, cardiac outputs and myocardial oxygen consumption rates were measured, and immediately thereafter 5 ml aliquots of perfusion fluid were sampled for estimation of the initial perfusate substrate and sorbitol concentrations. The system was now considered "closed" (section 5.3B).

Hearts were perfused for a further 65 minutes. Aortic outputs and coronary flow rates were measured at 15, 30 and 45 minutes, and at 30 minutes a total of 1 ml perfusate was withdrawn for estimation of myocardial oxygen consumption rates. At 35 min, 50 µl of radioactively-labelled ^{3}H-sorbitol solution was added to the perfusion fluids for the calculation of the circulating volume.

Sixty five minutes after left atrial perfusion had commenced, further 5 ml perfusion fluid aliquots were taken for calculation of myocardial metabolic data. Final measurements of aortic outputs, rates of coronary flows and myocardial oxygen consumption were then made.

Experiments were terminated after 70 minutes' working heart perfusions by rapidly removing the hearts from the cannulae. Left and right ventricular tissue was then prepared for the measurement of glycogen contents and of dry weights by the methods described in Appendix 1.D. Data for heart metabolism during these experiments were calculated according to the equations described in Appendix 1.F.

In a separate series of experiments, hearts from trained and control animals were clamped in Wollenberger tongs precooled in liquid nitrogen after 15 minutes non-working, Langendorff perfusions. Myocardial levels of ATP, PCr, glycogen, citrate, cyclic AMP and cyclic GMP were determined by the methods described in Appendix 1.D. The mean tissue glycogen level measured in this group of hearts, was taken as the value for G_o in the appropriate equations in Appendix 1.F.

B. Results.

Body weights, left and right ventricular dry weights, and resting heart rates in trained and control rats.

Left and right ventricular dry weights were not different between trained and control rats, but trained rats had significantly lower body weights and slower resting heart rates measured under light anaesthesia (Table 6.1). Table 6.1 also includes body and heart weight data from rats studied in those experiments described in subsequent sections.

The finding that heart weights were not increased in trained rats is compatible with those studies showing that the heart weights of male rats subjected to a programme of treadmill running are not invariably greater than those of control rats (section 2.3A). The reduction in heart rates measured in trained rats under light anaesthesia is compatible with the resting bradycardia of training. The mean 35 beats/min heart rate reduction in trained rats is of the order reported by other workers (section 2.3G).

TABLE 6.1

BODY WEIGHTS, HEART WEIGHTS AND RESTING HEART RATES OF TRAINED AND CONTROL RATS.

	BODY WEIGHTS (g)	LEFT VENTRICULAR DRY WEIGHTS (g)	RIGHT VENTRICULAR DRY WEIGHTS (g)	HEART RATES UNDER LIGHT ANAESTHESIA (beats/min)
Trained rats	296 ±3 (107)	0,13 ±0,01 (92)	0,04 ±0,01 (58)	398 ±8 (22)
Control rats	313 ±2 (105)	0,13 ±0,01 (68)	0,04 ±0,01 (42)	434 ±4 (25)
P value	$P < 0,01 \times 10^{-4}$	NS	NS	$P < 0,0005$

Results are expressed as mean ± SEM (number of measurements).

Note that both body weights and heart rates under light anaesthesia are significantly lower in trained rats, but heart weights are unaltered by training.

Myocardial contents of ATP, PCr, glycogen, citrate, cyclic AMP and cyclic GMP measured in hearts of trained and control rats after 15 minutes' Langendorff, non-working heart perfusion.

Table 6.2 shows that ATP, PCr, glycogen, citrate and cyclic GMP levels were not different in hearts from trained and control rats after 15 minutes' Langendorff, non-working heart perfusion. Cyclic AMP levels were,however,significantly lower in hearts from trained animals.

The findings that myocardial ATP, PCr and glycogen levels were not different between trained and control hearts is consistent with the published literature,as is the finding that myocardial glycogen levels were not different between trained and control animals that had not fasted (section 2.3B). This latter finding adds further evidence to Segel's contention[157] that training does not increase myocardial glycogen levels per se, but that it may alter the myocardial glycogen synthetic response to fasting.

Lower cyclic AMP levels in hearts from trained animals are consistent with the findings of Kleitke, Wollenberger, Krause et al[226] who reported reduced cyclic AMP levels in hearts of swimming-trained rats.

Cardiac outputs, stroke volumes, and rates of coronary flow and myocardial oxygen consumption of hearts perfused with the 6 different substrate combinations.

When heart rates, atrial filling pressures and aortic column heights were kept constant at levels previously shown to produce peak heart function, there were no differences in gross heart function, measured as cardiac outputs, and rates of either coronary flow or myocardial oxygen

TABLE 6.2

MYOCARDIAL ATP, PCr, GLYCOGEN, CITRATE, CYCLIC AMP AND CYCLIC GMP LEVELS MEASURED IN HEARTS FROM TRAINED AND CONTROL RATS AFTER 15 MINUTES' LANGENDORFF, NON-WORKING HEART PERFUSION.

UNITS	ATP µmol/g	PCr µmol/g	GLYCOGEN µmol glucose equiv/g	CITRATE µmol/g	CYCLIC AMP nmol/g	CYCLIC GMP nmol/g
Trained rats	3,89 ±0,07 (9)	4,47 ±0,45 (9)	9,13 ±0,57 (8)	0,20 ±0,04 (9)	0,21 ±0,02 (9)	0,03 ±0,01 (9)
Control rats	3,83 ±0,14 (8)	5,10 ±0,58 (8)	9,87 ±0,92 (9)	0,21 ±0,05 (8)	0,28 ±0,03 (7)	0,03 ±0,01 (7)
P Value	NS	NS	NS	NS	< 0,03	NS

Results are expressed as Mean ± SEM (number of measurements).

Note that only cyclic AMP levels are significantly altered by training.

consumption between hearts from trained and control animals perfused with glucose (Fig. 6.1), glucose-insulin (Fig. 6.2), lactate (Fig. 6.3), palmitate (Fig. 6.4) or palmitate-glucose-insulin (Fig. 6.5). Coronary flow rates were, however, significantly lower in trained hearts perfused with glucose-lactate-insulin (Fig. 6.6). When heart rates were increased to 390 beats/min there were again no significant differences in any of these parameters between trained and control hearts perfused with glucose-insulin (Fig. 6.7).

These data are at variance with those reported by Scheuer and his colleagues for hearts from both swimming- and running-trained rats (Section 2.3F). It should, however, be noted that these workers did not use the same steady-state perfusion protocols as were used in these experiments. In their experiments, hearts were perfused under conditions of changing atrial filling pressures and with isovolumic beats interspersed at the different atrial filling pressures.

Substrate metabolism during steady state working heart perfusions.

The substrate metabolism of hearts from trained and control animals during the 1-hour steady state perfusions with the different substrate combinations is compared in Table 6.3. Although no significant differences were noted, the release of lactate was lower in trained hearts perfused with glucose, glucose-insulin and palmitate-glucose-insulin. Glucose uptakes, calculated glycolytic fluxes and calculated glucose oxidation rates were also higher in trained than in control hearts, when perfused with palmitate-glucose-insulin.

As reviewed in section 2.3C, Scheuer, Penpargkul and Bhan[249] also found no differences in the metabolism of radioactively-labelled ^{14}C-palmitate and ^{14}C-glucose by potassium-arrested, isolated Langendorff perfused non-working hearts from swimming-trained and control rats,

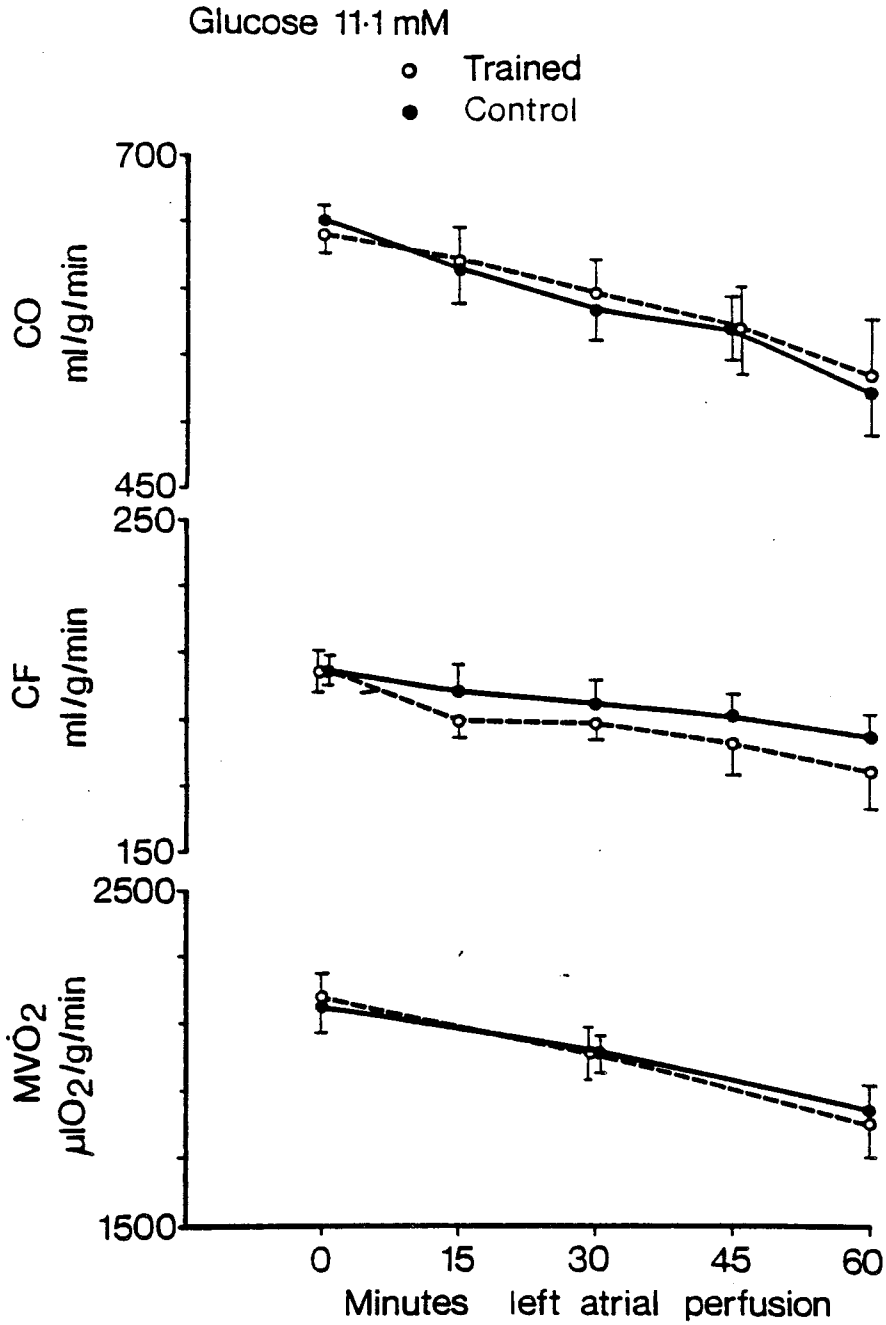

Figure 6.1

Comparison of mechanical performance of hearts from trained and control rats perfused with glucose.
Abbreviations as for Figure 5.2

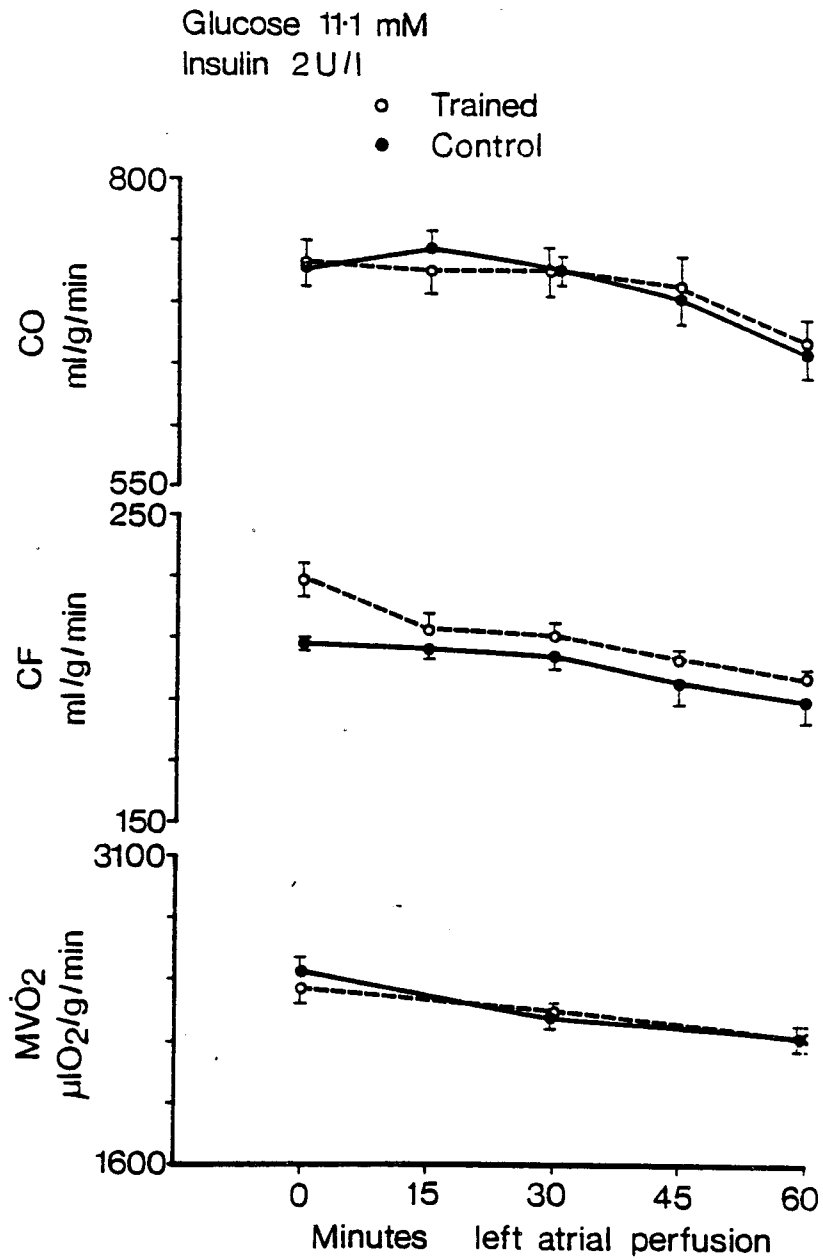

Figure 6.2

Comparison of mechanical performance of hearts from trained and control rats perfused with glucose-insulin.
Abbreviations as for Figure 5.2

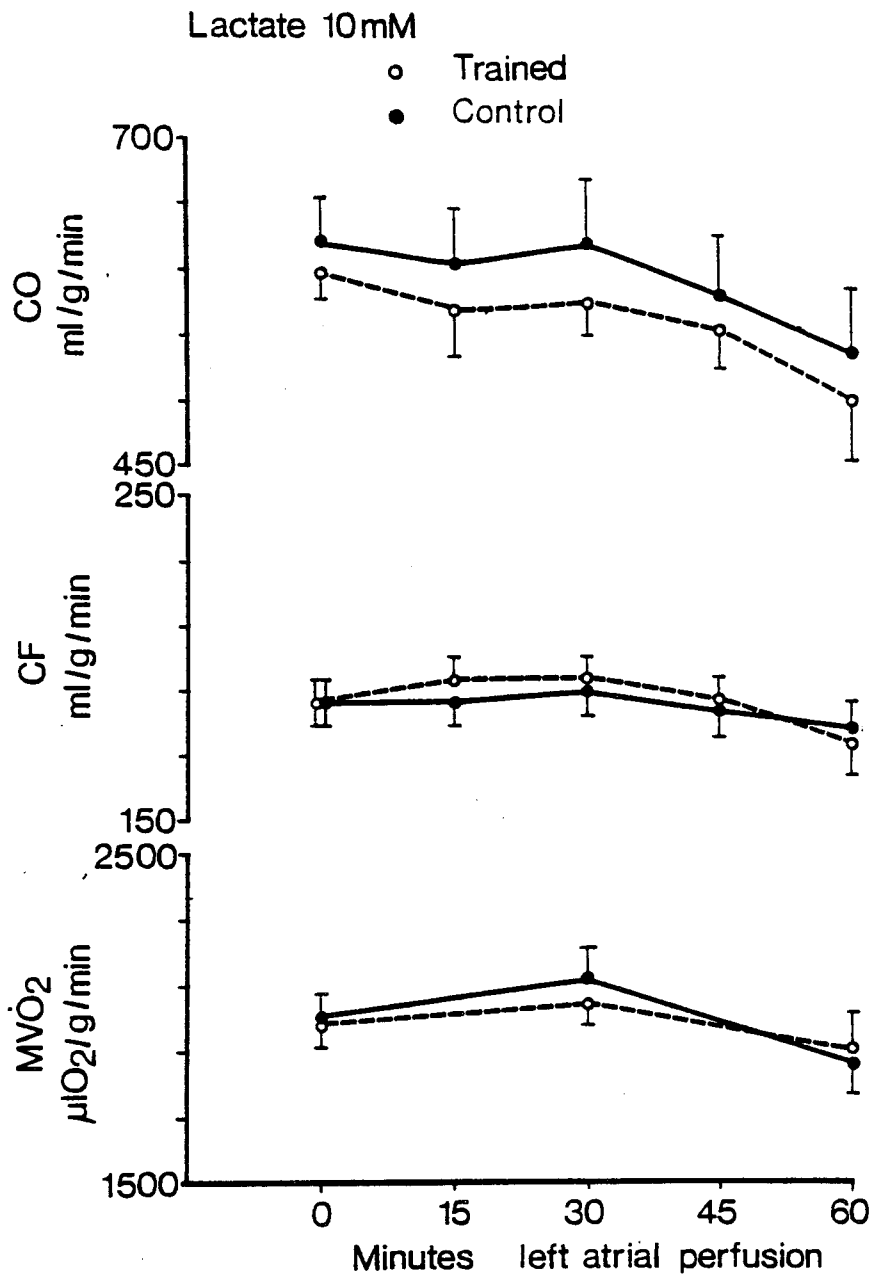

Figure 6.3

Comparison of mechanical performance of hearts from trained and control rats perfused with lactate.
Abbreviations as for Figure 5.2

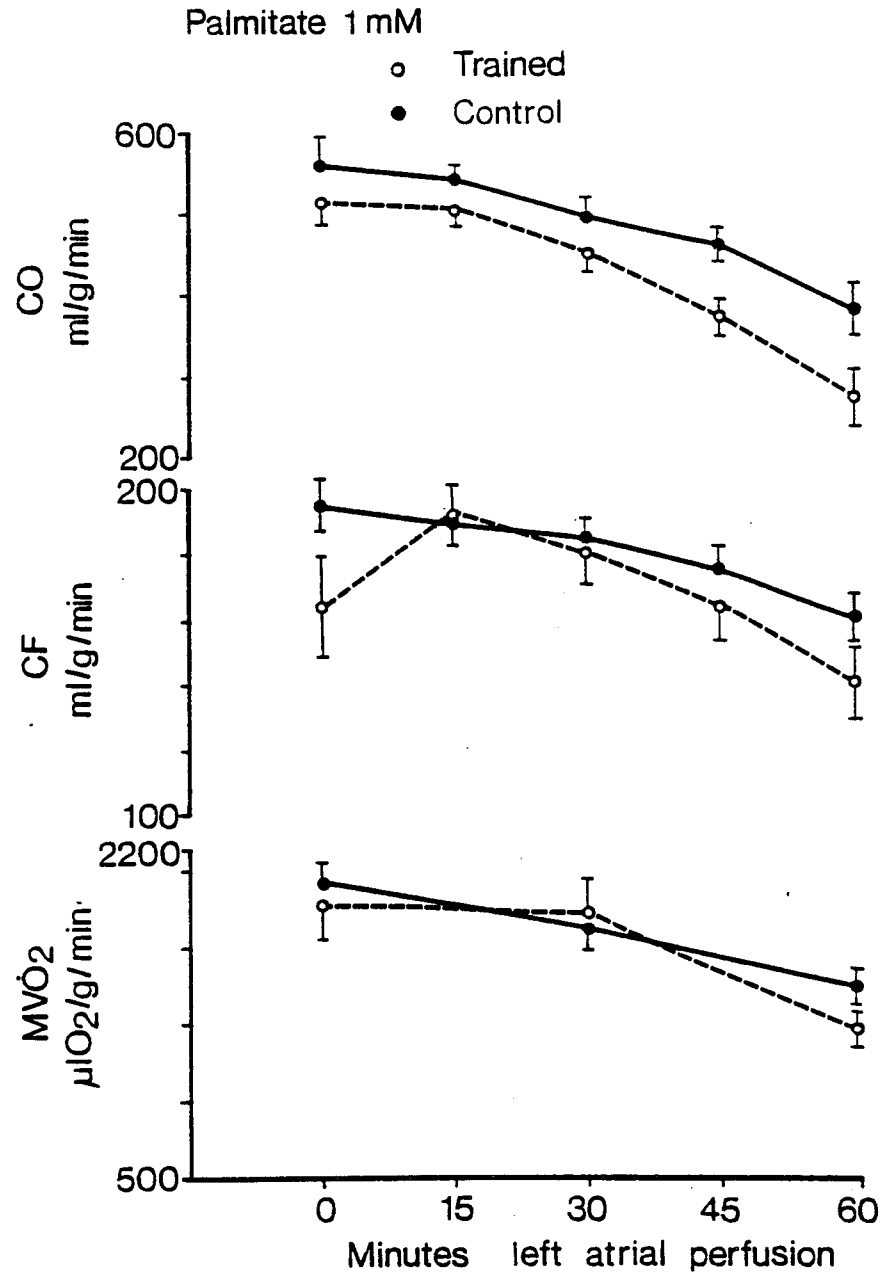

Figure 6.4

Comparison of mechanical performance of hearts from trained and control rats perfused with palmitate.
Abbreviations as for Figure 5.2

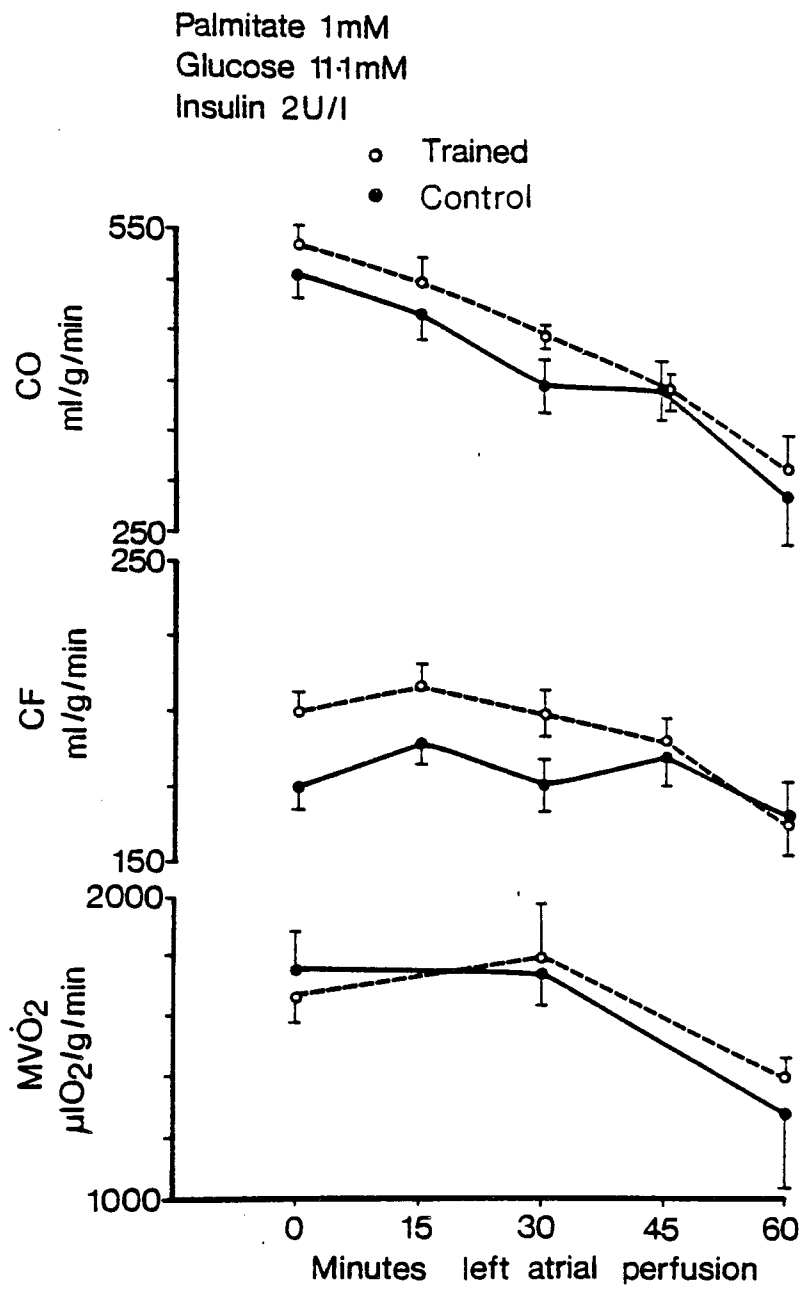

Figure 6.5

Comparison of mechanical performance of hearts from trained and control rats perfused with palmitate-glucose-insulin.
Abbreviations as for Figure 5.2

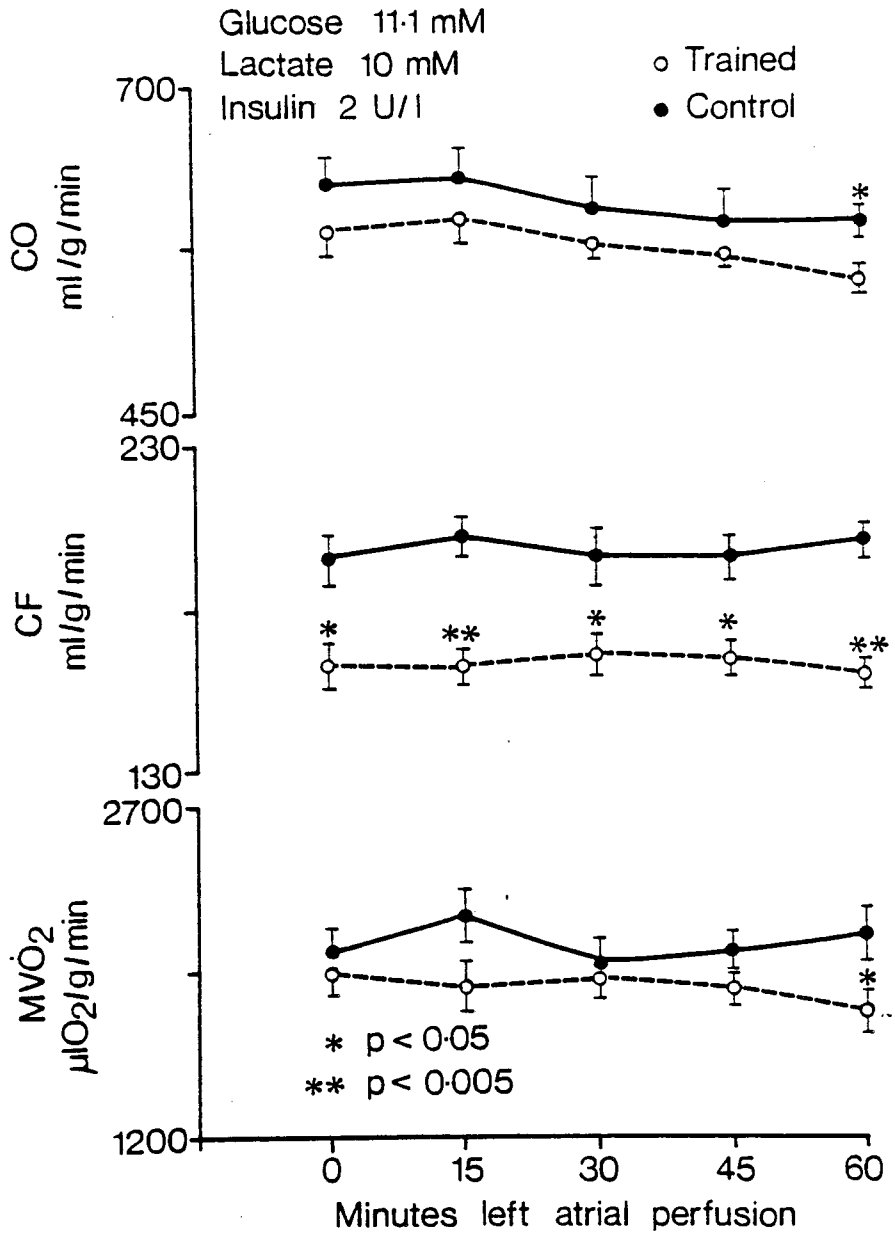

Figure 6.6

Comparison of mechanical performance of hearts from trained and control rats perfused with glucose-lactate-insulin. Note that coronary flow rates are significantly lower in trained hearts.
Abbreviations as for Figure 5.2

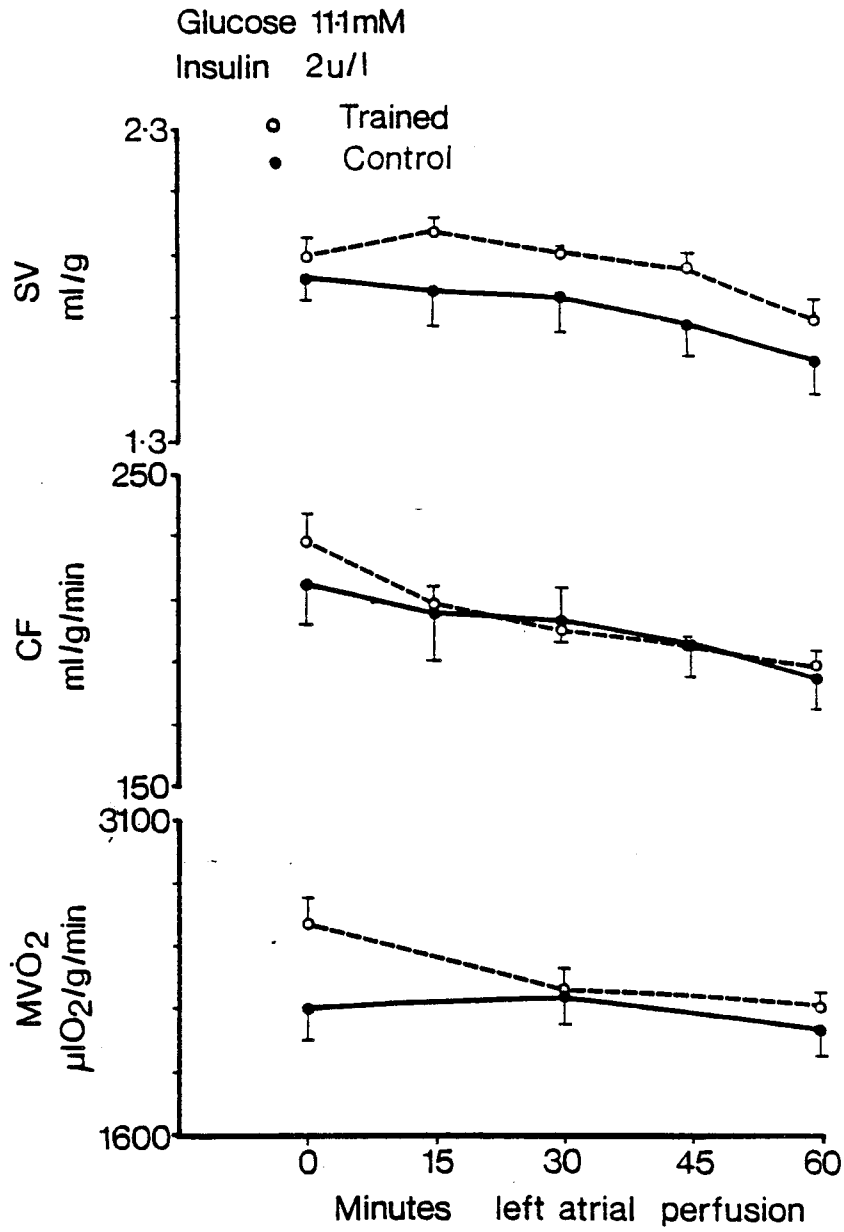

Figure 6.7

Comparison of mechanical performance of hearts from trained and control rats perfused with glucose-insulin at heart rates of 390 beats/min.
Abbreviations as for Figure 5.2

TABLE 6.3

SUBSTRATE METABOLISM OF HEARTS FROM TRAINED AND CONTROL RATS DURING 60 MINUTE STEADY STATE PERFUSIONS WITH DIFFERENT SUBSTRATE COMBINATIONS.

JBSTRATE COMBINATION	TRAINING STATUS (No. of hearts)	SUBSTRATE UPTAKE µmol/g/min	GLYCOGEN UTILIZATION µmol glucose equiv/g/min	GLYCOLYTIC FLUX µmol glucose equiv/g/min	GLYCOLYTIC ATP PRODUCTION µmol/g/min	LACTATE RELEASE µmol/g/min	CE OXIDATION µmol/g/min
l,1 mM Glucose	Trained rats (6)	3,20 ±0,09	0,10 ±0,01	3,29 ±0,09	6,69 ±0,19	0,26 ±0,11	3,17 ±0,12
	Control rats (7)	3,49 ±0,31	0,12 ±0,01	3,61 ±0,31	7,29 ±0,63	0,30 ±0,09	3,46 ±0,34
.,1 mM Glucose ısulin 2 U/L	Trained rats (6)	5,04 ±0,38	-0,03 ±0,01	5,01 ±0,38	10,00 ±0,76	1,03 ±0,08	4,50 ±0,37
	Control rats (7)	5,40 ±0,26	-0,01 ±0,01	5,40 ±0,26	10,50 ±0,48	1,39 ±0,15	4,70 ±0,23
mM Palmitate % Albumin)	Trained rats (7)	0,76 ±0,03	0,09 ±0,01	0,09 ±0,01	0,26 ±0,04	0,18 ±0,15	-0,01 ±0,08
	Control rats (7)	0,73 ±0,05	0,07 ±0,01	0,09 ±0,01	0,22 ±0,04	-0,01 ±0,20	0,03 ±0,08
mM Palmitate % Albumin) ,1 mM Glucose sulin 2 U/L	Trained rats (6)	Glucose 2,73 ±0,32 Palmitate 0,55 ±0,07	0,001 ±0,01	2,72 ±0,33	5,45 ±0,66	1,15 ±0,15	2,15 ±0,33
	Control rats (7)	Glucose 1,98 ±0,45 Palmitate 0,61 ±0,10	0,02 ±0,01	2,00 ±0,46	4,01 ±0,92	1,56 ±0,15	1,22 ±0,42
mM Lactate	Trained rats (6)	6,87 ±0,37	0,1 ±0,01	0,11 ±0,01	0,33 ±0,02	-	0,33 ±0,02
	Control rats (7)	7,02 ±0,66	0,11 ±0,01	0,11 ±0,01	0,33 ±0,03	-	0,33 ±0,03

sults are expressed as mean ± SEM.

although calculated turnover rates in the triglyceride pool were higher in trained hearts. However, Moreau, Guilland, Athias et al[250] have reported that the oxidation rates of both radioactively-labelled palmitate and of endogenous triglycerides were significantly higher in hearts from swimming-trained rats, even under less severe perfusion conditions (atrial filling pressures 10 cmH_2O, aortic column height 80 cms, unpaced heart rates). It should be noted that in that study, trained hearts also performed more work.

Additional evidence that will be presented below suggests that the metabolic adaptations to exercise training may only be revealed by these more precise radioactive techniques.

6.4 COMPARISON OF LEFT VENTRICULAR FUNCTION OF HEARTS FROM TRAINED AND CONTROL RATS UNDER CONDITIONS OF CHANGING HEART RATES AND ATRIAL FILLING PRESSURES.

Experimental protocol.

As the results of the above studies were at variance with the work of Scheuer and his colleagues, I considered whether the choice of a perfusion protocol involving only steady-state work as compared to their changing workload protocol, could explain our different findings. Therefore, in the series of experiments reported in this section, hearts were perfused under those conditions described in Section 5.3B, of changing atrial filling pressures and heart rates, with isovolumic beats interspersed, and with a left ventricular catheter *in situ*. Two different substrate combinations were used, namely:

11,1 mM D(+) glucose plus insulin at 2 u/litre; and
1 mM palmitate bound to 3% albumin and 11,1 mM D(+) glucose plus insulin at 2 u/litre.

Results.

The results of these experiments are presented graphically in Figures 6.8 and 6.9.

Figure 6.8 shows that when perfused with glucose-insulin, under conditions of changing atrial filling pressures and heart rates and with isovolumic beats interspersed, stroke volumes and rates of both coronary flow and myocardial oxygen consumption were significantly higher in hearts from trained rats at all heart rates, when the atrial filling pressure was 25 cmH_2O or more. Left ventricular max +ve or max -ve dP/dt values and left ventricular end diastolic pressures were however not different between the 2 groups of hearts.

These data are therefore in accord with the work of Schaible and Scheuer[262] who also found that under conditions of changing atrial filling pressures and heart rates, and with a left ventricular catheter *in situ*, stroke volumes were significantly higher in running-trained than in control hearts without there being significant differences in either LV max +ve or max -ve dP/dt values. It should be noted that in this study, but not in that of Schaible and Scheuer, rates of myocardial oxygen consumption and coronary flow were also significantly higher in hearts from running-trained rats. Peak left ventricular pressures were also much greater in this than in the study of Schaible and Scheuer (178 vs 105 mmHg at 20 cmH_2O atrial filling pressure) due to the higher aortic column heights used in this study. It is possible that had Schaible and Scheuer used workloads similar to these in their study, the higher rates of coronary flow and myocardial oxygen consumption found in hearts from running-trained rats might also have achieved statistical significance. It is not clear how other differences in perfusion protocols, namely the lower glucose and lower free calcium

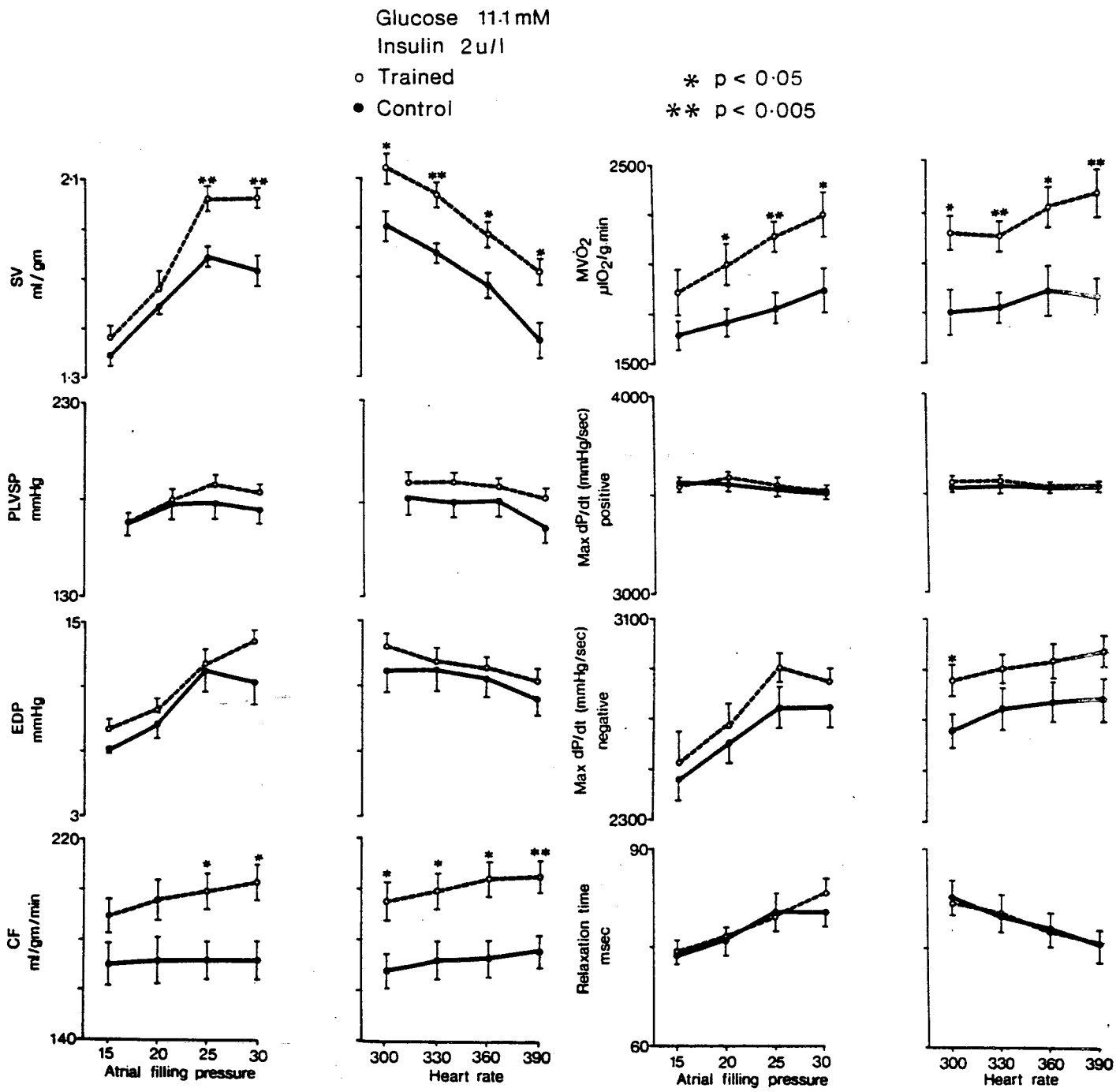

Figure 6·8

Comparison of left ventricular function of hearts from trained and control rats perfused with glucose - insulin under conditions of changing atrial filling pressures and heart rates. Note that stroke volumes and rates of coronary flow and myocardial oxygen consumption are increased in trained hearts.

Abbreviations as for Figure 5·5

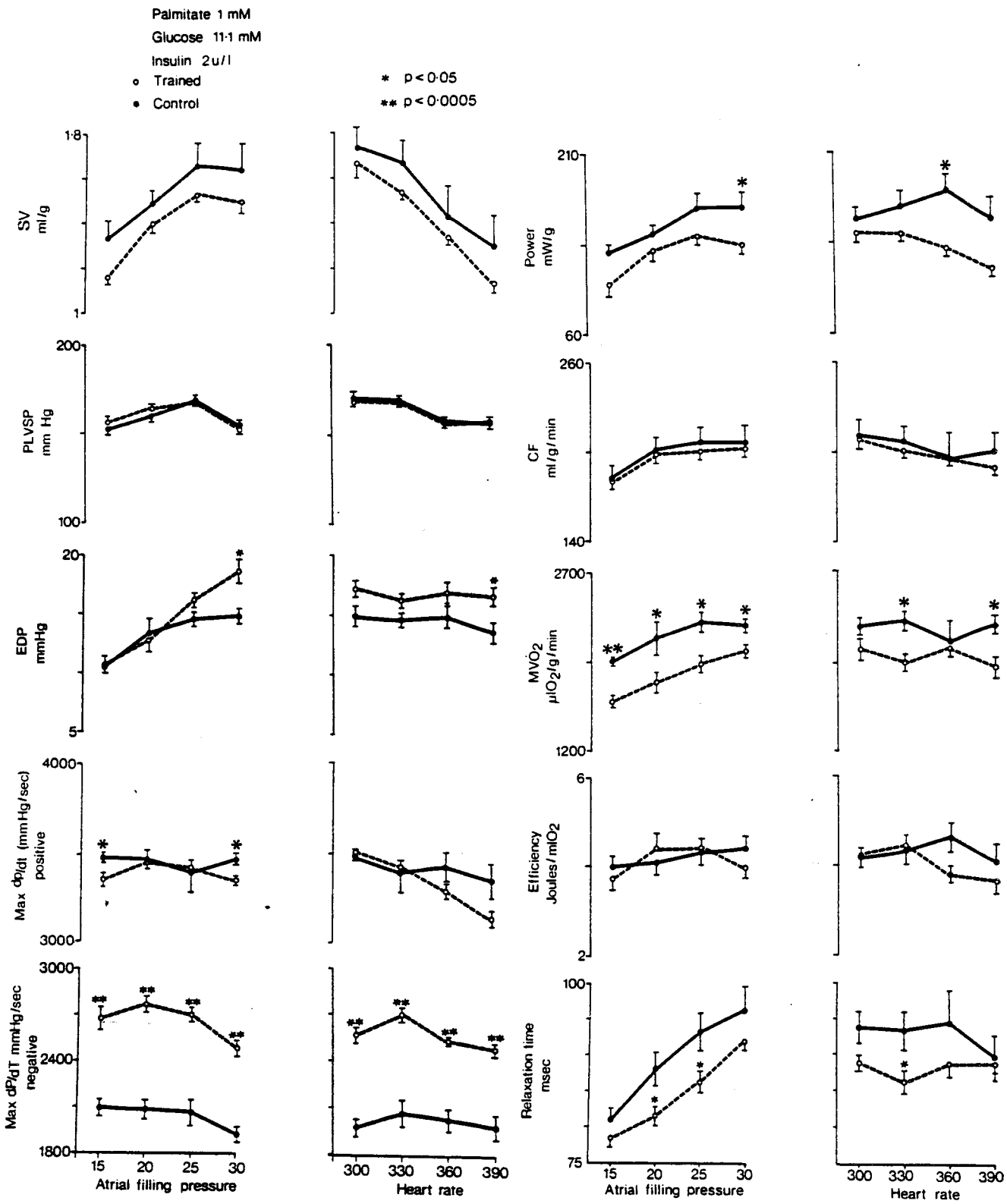

Figure 6·9

Comparison of left ventricular function of hearts from trained and control rats perfused with palmitate-glucose-insulin under conditions of changing atrial filling pressures and heart rates. Note that LV max-ve $^{dp}/_{dt}$ values are greater, and relaxation times shorter in trained hearts. Rates of myocardial oxygen consumption were increased in control hearts. Abbreviations as for Figure 5·5

concentrations used by Schaible and Scheuer, may have influenced the comparison between our respective studies. In section 6.5, further consideration is given to the role of the perfusate free calcium concentration in demonstrating differences in mechanical performance of hearts from trained and control animals.

There are 3 possible explanations why the greater stroke volumes of hearts from trained animals were not associated with higher LV max +ve dP/dt values either in this study or that of Schaible and Scheuer, namely: (i) Either the recording apparatus was insensitive to small changes in L.V. max +ve dP/dt, or (ii) there were differences in the mean L.V. +ve dP/dt values which were not detected in the absence of changes in maximum L.V. +ve dP/dt values, or (iii) the greater stroke volumes in the trained hearts were due to greater end-diastolic volumes (Frank/Starling mechanism) that were not detected by measurements of left ventricular pressures either because of insensitive equipment or because training has altered the left ventricular pressure/volume relationship (left ventricular compliance). Alternatively, as suggested by Schaible and Scheuer, the increased stroke volumes found in trained hearts may be due to alterations in fibre shortening rates (as directly computed by these workers) without there being equivalent changes in left ventricular pressure development phenomena.

In section 6.5 evidence will be presented which shows that the recording apparatus used in these experiments was not optimal and may have been too insensitive to measure differences in either L.V. max +ve or max -ve dP/dt values between the 2 groups of hearts.

When hearts from trained and control rats were perfused with palmitate-glucose-insulin, the major difference in left ventricular function was that the rates of relaxation were significantly greater and relaxation times shorter in trained hearts (Fig. 6.9). The significantly higher

myocardial oxygen consumption rates and greater power outputs of control hearts is misleading because, in those experiments, 2 control hearts were unable to produce cardiac outputs at the higher atrial filling pressures and heart rates, and data from those 2 experiments were not included in these figures.

The finding that trained hearts had greater rates of myocardial relaxation and higher rates of myocardial glycolytic ATP production when perfused with palmitate-glucose-insulin (Table 6.3) is in accord with the postulated relationship between glycolysis and myocardial relaxation (section 5.3). The measurement of glycolytic ATP production rates using the ^{3}H-glucose method would be necessary to determine whether these higher glycolytic rates in trained hearts were statistically significant. If so, this would indicate that the normal inhibitory action of palmitate on myocardial glycolysis is reduced by exercise training.

6.5 COMPARISON OF LEFT VENTRICULAR FUNCTION, METABOLISM, CONTRACTILE PROTEIN ATP HYDROLYZING ACTIVITIES AND PHOSPHORYLATION LEVELS OF HEARTS FROM TRAINED AND CONTROL RATS AFTER VARYING PERIODS OF ISOPROTERENOL INFUSION.

The basic protocol used in all these studies was the same as that described in section 5.3, with the exception that the experiments were terminated after varying periods of isoproterenol infusion.

Two different experimental studies were performed. In the first, the left ventricular function of hearts from trained and control rats was measured with the same paper recorder system that had been used in those studies reported in the previous chapter (section 5.3B). In these experiments, myocardial metabolite levels and absolute glycolytic rates

were also measured, the latter by the ^{3}H-glucose method. In the second group of experiments, instantaneous left ventricular pressures and aortic flows were measured and computed on-line with a minicomputer and stored for later analysis. The perfusion and recording apparatus used in these experiments has been described in section 5.2C. Data reduction was achieved by the methods described in Appendix 1.F. In these experiments, which were performed at 3 different perfusate calcium concentrations, myocardial contractile protein ATP hydrolyzing activities and phosphorylation levels, and myocardial cyclic AMP contents were measured at various times after the commencement of isoproterenol infusion.

A. Studies of left ventricular function, glycolytic rates and tissue metabolite levels.

Experimental protocol.

In these experiments which were performed on Wistar-Weissman rats, the perfusion protocol was identical to that described in section 5.3 (Fig. 5.7). Left ventricular function was measured during 15-minute control left atrial perfusions, followed by 25-minute perfusions during which 6,5 x 10^{-7}M isoproterenol was infused. In a separate series of experiments, glycolytic rates were measured by the ^{3}H-glucose method (Appendix 1.C) in hearts perfused without left ventricular catheters. These experiments were terminated by clamping the hearts in pre-cooled Wollenberger tongs. These hearts were then extracted and their tissue contents of ATP, PCr, glycogen, citrate and cyclic AMP measured. In a final series of experiments, the heart rates of unpaced hearts from trained and control rats was measured 4 minutes after the isoproterenol infusion had begun. These hearts were perfused in the absence of a left ventricular catheter.

Results.

When steady state conditions were maintained with a left ventricular catheter in situ, there were no differences between hearts from trained and control animals in stroke volumes, peak left ventricular systolic pressures or end-diastolic pressures, ejection times, L.V. max +ve and max -ve dP/dt values, relaxation times, calculated heart work, efficiency, and rates of coronary flow or myocardial oxygen consumption (Fig. 6.10). However, within 5 minutes of beginning the isoproterenol infusion, stroke volumes and L.V. max +ve and max -ve dP/dt values were all significantly higher in the trained hearts. Peak left ventricular pressures, calculated heart work, rates of coronary flow and myocardial oxygen consumption were also higher in trained hearts, but these differences did not achieve statistical significance.

The greater L.V. max +ve dP/dt values from the same end-diastolic pressures provide strong evidence for enhanced myocardial contractility in hearts from trained rats during β-stimulation with isoproterenol. This enhanced inotropic response to β-stimulation occurred independently of an altered chronotropic response, because the heart rate responses of unpaced hearts (in a different series of experiments) to $6{,}5 \times 10^{-7}$M isoproterenol infusion were not different between trained and control hearts. Four minutes after the isoproterenol infusion had begun, the rates of hearts from trained rats had increased to 376 ± 8 (Mean ± SEM for 20 hearts) and to 390 ± 8 (Mean ± SEM for 20 hearts) in untrained hearts.

Glycolytic rates were significantly higher in trained hearts both before and during isoproterenol infusion (Fig. 6.11), whereas after 25 minutes infusion trained hearts had significantly lower PCr and cyclic AMP levels (Table 6.4).

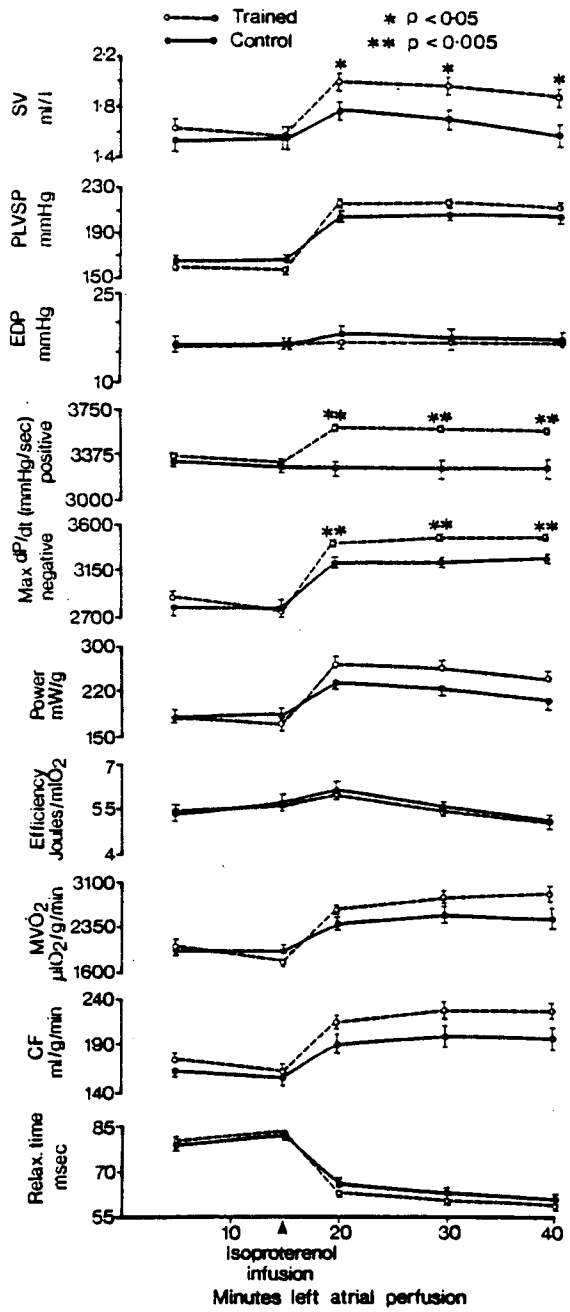

Figure 6·10

Comparison of effects of $6{\cdot}5 \times 10^{-7}$M isoproterenol infusion on left ventricular function of hearts from trained and control rats.
Note that after isoproterenol infusion, trained hearts develop significantly greater stroke volumes, max +ve and max -ve dp/dt values.
Each point on the graph represents data for at least 7 experiments.
Abbreviations as for Figure 5·5

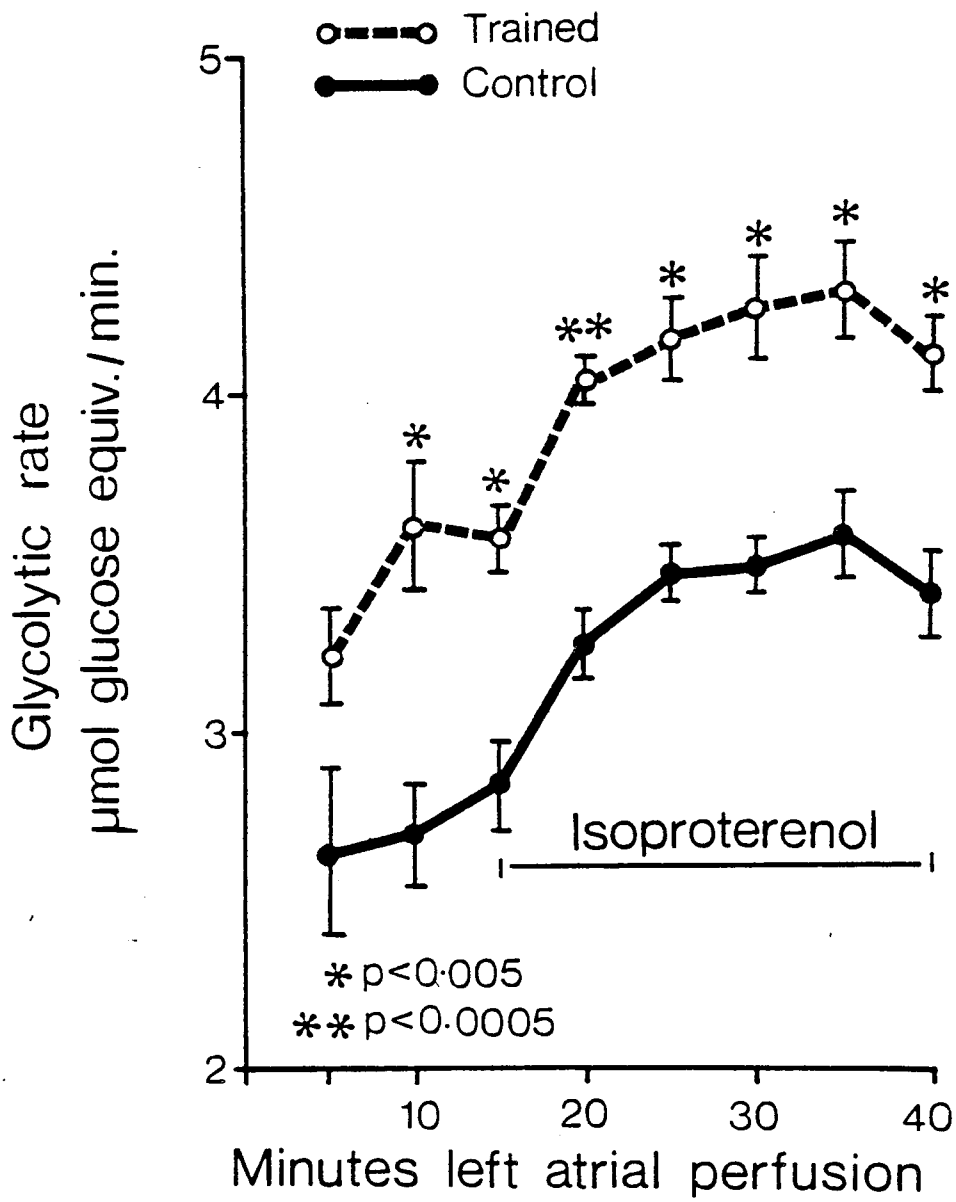

Figure 6.11

Absolute glycolytic rate of hearts from trained and control rats before and during $6{\cdot}5 \times 10^{-7}$ M isoproterenol infusion.
Note that glycolytic rates are significantly higher in trained hearts.

TABLE 6.4

TISSUE ATP, PCr, GLYCOGEN, CITRATE AND CYCLIC AMP LEVELS IN HEARTS FROM TRAINED AND CONTROL RATS AFTER 25 MINUTES' $6,5 \times 10^{-7}$M ISOPROTERENOL INFUSIONS AT PERFUSATE $CaCl_2$ CONCENTRATIONS OF 2,2 mM.

UNITS	ATP µmol/g	PCr µmol/g	GLYCOGEN µmol glucose equiv/g	CITRATE µmol/g	CYCLIC AMP nmol/g
Trained rats	3,12 ±0,09 (7)	2,68 ±0,18 (7)	8,82 ±0,55 (7)	0,12 ±0,01 (7)	0,56 ±0,04 (7)
Control rats	2,96 ±0,09 (14)	3,75 ±0,14 (14)	8,89 ±0,46 (14)	0,12 ±0,01 (13)	0,75 ±0,03 (11)
P value	NS	< 0,0005	NS	NS	< 0,005

Note that levels of PCr and cyclic AMP are significantly lower in trained hearts.

Values are Mean ± SEM (number of measurements).

B. Studies of left ventricular function, ATP hydrolyzing activities, phosphorylation levels and cyclic AMP contents after varying periods of isoproterenol infusion at 3 different perfusate calcium concentrations.

Experimental protocol.

In these experiments, which were performed on Long-Evans rats, hearts were perfused according to the same protocol used in the previous sections, with the exception that special attention was paid to left ventricular performance and biochemical changes during the first 5 minutes of isoproterenol infusion. The effect of different perfusate $CaCl_2$ concentrations was also studied.

Left ventricular function and aortic flows were measured by the minicomputer (section 5.2C) 5 and 15 minutes after left atrial perfusion had commenced and at 15, 35, 60, 120, 180, 240 and 300 seconds after commencement of $6,5 \times 10^{-7}$M isoproterenol infusion. Coronary flow rates were measured manually by collecting 15-second samples of coronary effluent at these time intervals.

Depending on the experimental protocol, the perfusions were terminated at varying times either immediately before (control) or after isoproterenol had been infused for 15, 30, 60, 120, 180 or 300 seconds. Hearts were either clamped in Wollenberger tongs at these time intervals and later extracted for measurement of their cyclic AMP levels, or were manually removed from the perfusion apparatus and their contractile protein ATP hydrolyzing activities and phosphorylation levels assayed according to the methods of Resink and Gevers[482,483] (Appendix 1.D).

All experiments were performed at 3 different $CaCl_2$ concentrations - 1,6, 2,2 and 3,6 mM. The perfusate calcium and magnesium

concentrations were altered in tandem so that a constant Ca^{2+}/Mg^{2+} ratio was maintained. The free calcium levels measured, at the three $CaCl_2$ concentrations, with the calcium electrode (Appendix 1.C) were 1,3, 1,9 and 3,3 mM respectively.

Results.

The left ventricular function of hearts from trained and control rats perfused at the 3 different calcium concentrations is shown in Figures 6.12 - 6.14. These studies were done principally to evaluate the effects of training on the biochemical response of the contractile protein ATPases to isoproterenol infusion. Thus, complete left ventricular performance data is not available for each time point at each perfusate calcium concentration. Nevertheless, sufficient data have been collected to allow specific conclusions to be drawn.

In accord with the previous findings, mechanical function was not different between trained and control hearts at any calcium concentration prior to isoproterenol infusion. It should be noted that in both groups, left ventricular performance, measured as cardiac output, coronary flow, peak left ventricular systolic pressure, ejection time, power and max +ve dP/dt, increases with increasing perfusate calcium concentration.

Figure 6.13 shows that at perfusate $CaCl_2$ concentrations of 2,2 mM, trained hearts developed significantly greater peak left ventricular systolic pressures, and L.V. max +ve and max -ve dP/dt, immediately after isoproterenol infusion. Cardiac outputs, rates of coronary flow and total power were insignificantly higher in trained hearts, whilst end-diastolic pressures and ejection times were not different between groups. These differences were still present after isoproterenol had been infused for 5 minutes. It is not clear why cardiac outputs failed to rise in

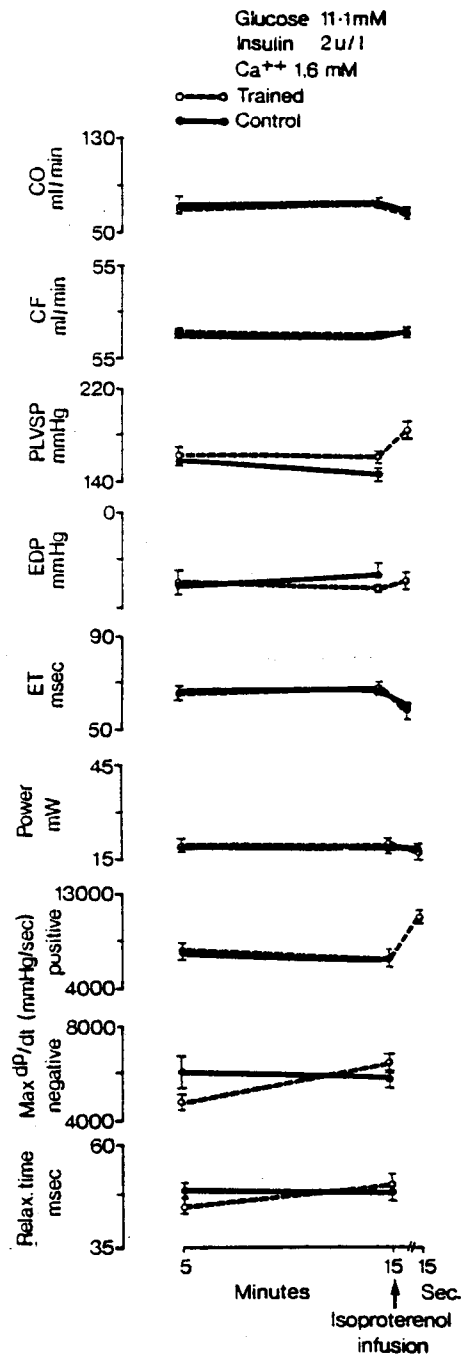

Figure 6·12

Comparison of left ventricular function of hearts from trained and control rats perfused at a $CaCl_2$ concentration of 1·6 mM.
Note that there are no differences in left ventricular function during control perfusions.
Each point represents data from at least 4 experiments.
Abbreviations as for Figure 5·5

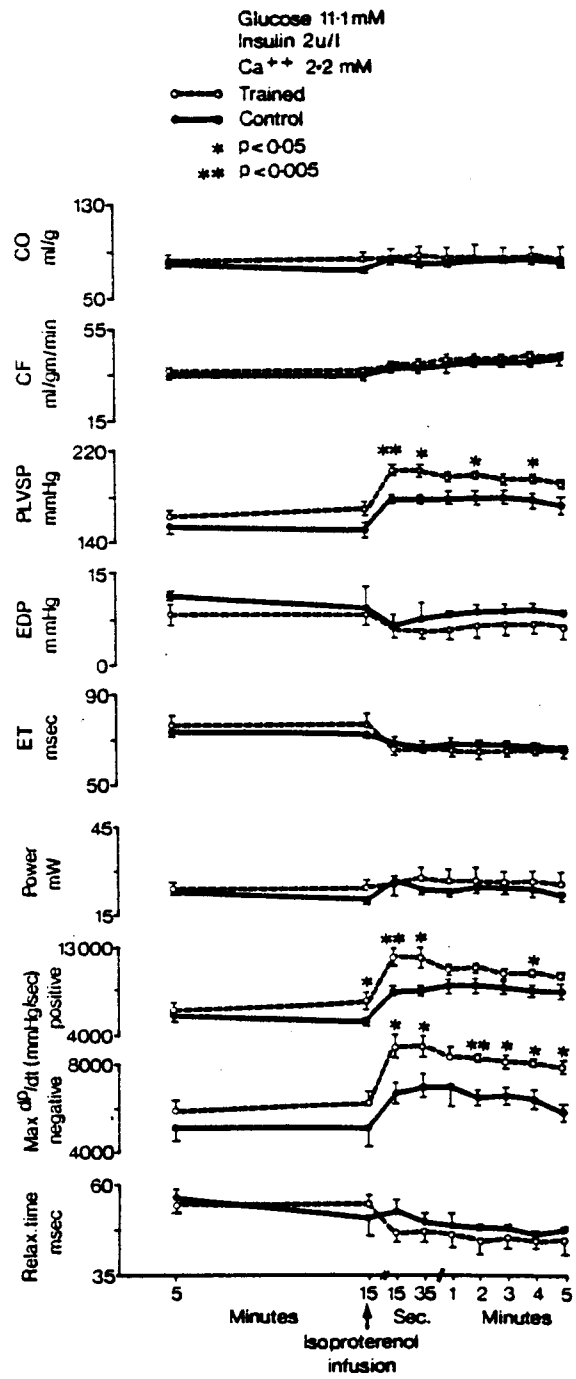

Figure 6·13

Comparison of effects of $6{\cdot}5 \times 10^{-7}$M isoproterenol infusion on left ventricular function of hearts from trained and control rats perfused at a $CaCl_2$ concentration of 2·2mM.

Note that trained hearts develop significantly greater peak left ventricular systolic pressures and max positive and max negative dp/dt values, immediately after isoproterenol infusion.

Each point represents data for at least 4 experiments.

Abbreviation as for Figure 5·5

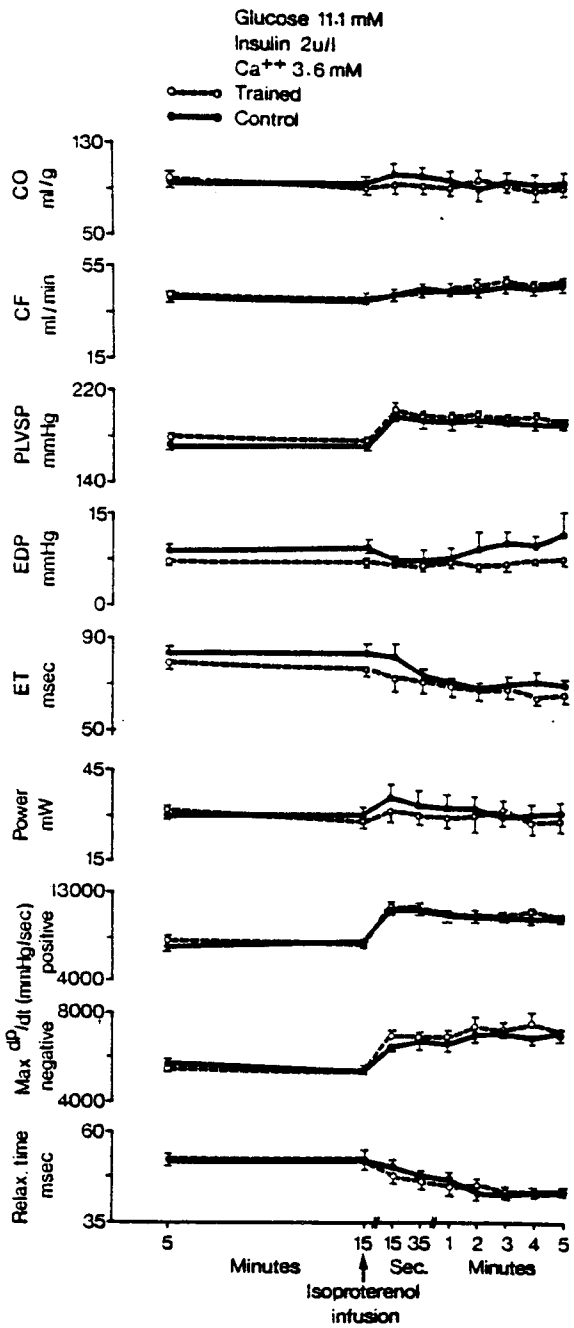

Figure 6·14

Comparison of effects of $6{\cdot}5 \times 10^{-7}$M isoproterenol infusion on left ventricular function of hearts from trained and control rats perfused at a $CaCl_2$ concentration of 3·6 mM.
Note that those differences in left ventricular function which were present at a $CaCl_2$ concentration of 2·2 mM (Figure 6·13), are abolished by increasing the perfusate $CaCl_2$ concentration.
Each point represents data from at least 4 experiments.
Abbreviations as for Figure 5·5

response to isoproterenol infusion, as was the case in the previous series of experiments (Fig. 5.7 and 6.10). In this series, end-diastolic pressures fell after isoproterenol infusion, whereas in the other 2 series, end-diastolic pressures rose after isoproterenol infusion. Whether or not this was due to the different rat species studied (Long-Evans vs Wistar Weissman), remains to be established.

Figure 6.14 shows that when perfused at $CaCl_2$ concentrations of 3,6 mM, the different left ventricular responses to isoproterenol infusion between trained and control hearts which were present at the lower $CaCl_2$ concentration were abolished. Thus, the superior heart function of trained rats was no longer apparent at the unphysiological calcium concentration. Stated differently, trained hearts achieved maximum left ventricular performance at physiological calcium levels and showed no further increase as the calcium concentration was increased, whereas control hearts reached equivalent maximum levels of heart function only when the calcium concentration was raised to unphysiological levels.

It should also be noted that the maximum rates of left ventricular pressure development increased in both trained and control hearts in response to isoproterenol infusion at all perfusate calcium concentrations. This indicates that the recording system used in the previous experiments was insufficiently sensitive to record changes in L.V. max +ve dP/dt values when control hearts were infused with isoproterenol (see Figures 5.7 and 6.10). It is also of interest that the L.V. max +ve dP/dt values measured in these experiments are the highest ever recorded for working rat hearts[458]. It is possible that values reported by other workers have also been limited by the frequency response characteristics of their recorders.

Actomyosin and myosin ATPase activities.

Table 6.5 lists the ATPase activities of natural actomyosin isolated from hearts of trained and control animals after 15 minutes' left atrial perfusions at $CaCl_2$ concentration of 1,6 mM. There were no differences in Ca^{++}-stimulated, Mg^{++}-dependent actomyosin ATPase activities, but myosin Ca^{++}-ATPase activities were significantly higher in trained hearts.

In response to isoproterenol infusion, the maximum velocity of myosin Ca^{++}-ATPase activity increased more rapidly and was significantly higher in hearts from trained animals (Fig. 6.15). At the higher $CaCl_2$ concentrations these differences between trained and control hearts were magnified (Table 6.6).

Actomyosin Ca^{++}/Mg^{++} activities decreased equally in response to isoproterenol infusions in both trained and control hearts at all perfusate calcium concentrations (data not shown).

Troponin-1 and myosin P light chain phosphorylation.

Isoproterenol stimulated troponin-1 phosphorylation at the same rate and to the same extent in hearts from trained and control rats, at all perfusate calcium concentrations. Troponin-1 phosphorylation occurs in response to increases in myoplasmic cyclic AMP levels so that the similar extents of troponin-1 phosphorylation between trained and control hearts are in accord with their similar initial cyclic AMP responses to isoproterenol infusions (Table 6.7).

In contrast, the rates and extents to which alkali-labile phosphate was incorporated into myosin P light chains were significantly greater in trained hearts after isoproterenol infusions (Fig. 6.16). These differences were magnified at higher perfusate calcium concentrations

TABLE 6.5

CONTRACTILE PROTEIN ATP-ASE ACTIVITIES OF NATURAL ACTOMYOSIN ISOLATED FROM HEARTS OF TRAINED AND CONTROL RATS AFTER 15 MINUTE WORKING HEART PERFUSIONS*

	ATP-ASE ACTIVITIES (nmol/mg/min)		
	ACTOMYOSIN	MYOSIN	
	(Ca^{++}/Mg^{++})	(Ca^{++})	(K^{+},EDTA)
Trained rats	156,4 ±10,0 (4)	322,5 ±2,4 (4)	129,6 ±3,3 (4)
Control rats	162,3 ±5,0 (4)	279,5 ±5,7 (4)	135,4 ±2,8 (4)
P value	NS	< 0,0005	NS

Note that trained hearts have significantly increased cardiac myosin Ca^{++}-ATPase activities.

* Data of Resink and Gevers, published here with their permission.

Values are Mean ± SEM (number of measurements).

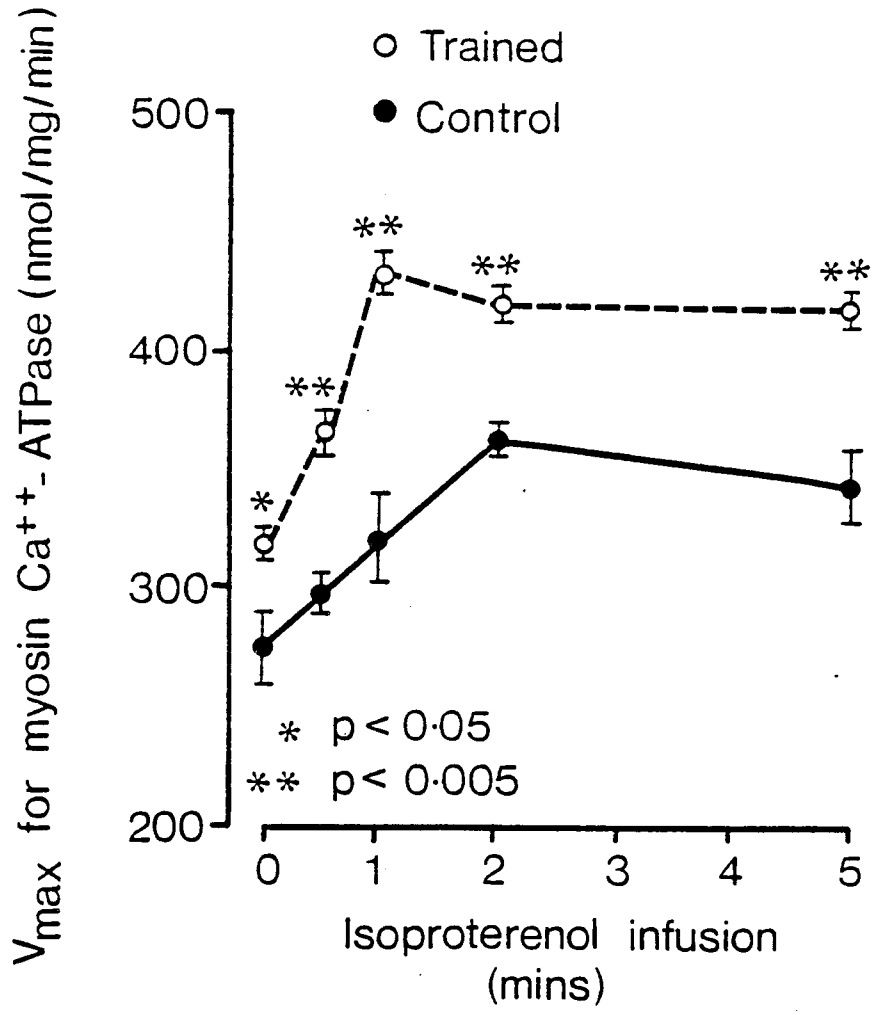

Figure 6·15

V_{max} of myosin Ca^{++}- ATPase of natural actomyosin isolated from perfused hearts of trained and control rats after varying periods of isoproterenol infusion.
Note that at all times ATPase activities are significantly higher in trained hearts.
Data of Resink and Gevers, published with permission.

TABLE 6.6

THE EFFECTS OF RUNNING TRAINING ON Vmax OF MYOSIN Ca^{++}-ATPase ACTIVITIES IN RESPONSE TO INCREASING PERFUSATE Ca^{++} CONCENTRATIONS AND/OR TREATMENT WITH $6,5 \times 10^{-7}$M ISOPROTERENOL.*

Vmax MYOSIN Ca^{++}-ATPase (nmol/mg/min)

Perfusate (Ca^{++}) mM	CONTROL RATS		TRAINED RATS	
	Control perfusion (A)	Isoproterenol infusion (1 min) (B)	Control perfusion (C)	Isoproterenol infusion (1 min) (D)
1,6	275 ±15	320 ±15	320* ±15	435++ ±5
2,2	265 ±15	323 ±6	361** ±9	524++ ±22
3,6	333 ±16	392 ±15	413** ±14	550++ ±18

* p <0,05 C vs A
** p <0,01 C vs A
++ p <0,001 D vs B

Note that myosin Ca^{++}-ATPase activities increase with increasing perfusate calcium concentrations, but that all values are significantly higher in hearts from trained animals.

* Data of Resink and Gevers, published here with their permission.

Values are Mean ± SEM for 4 experiments in each group.

TABLE 6.7

TISSUE CYCLIC AMP LEVELS (nmol/g) OF HEARTS FROM TRAINED AND CONTROL RATS PERFUSED AT TWO DIFFERENT CaCl2 CONCENTRATIONS FOR VARYING PERIODS OF 6,5 x 10^{-7}M ISOPROTERENOL INFUSION.

1,6 mM $CaCl_2$

TIME (SECONDS)	0*	15	30	60	120	180	300
Trained rats	0,37 ±0,04 (3)	-	0,80 ±0,19 (2)	0,91 ±0,13 (2)	1,08 ±0,19 (2)	-	1,44 ±0,39 (2)
Control rats	0,36 ±0,10 (2)	-	0,85 ±0,05 (3)	0,94 ±0,15 (2)	1,19 ±0,01 (2)	-	1,33 ±0,01 (2)

2,2 mM $CaCl_2$

Trained rats	0,39 ±0,06 (3)	0,88 ±0,2 (3)	0,90 ±0,05 (2)	0,94 ±0,05 (3)	-	0,83 ±0,04 (6)	-
Control rats	0,46 ±0,03 (3)	0,86 ±0,1 (3)	0,91 ±0,07 (3)	0,95 ±0,02 (3)	-	0,91 ±0,05 (6)	-

Note that there are no differences in cyclic AMP levels of hearts from trained and control rats at any time during isoproterenol infusion at either calcium concentration.

* Time 0 indicates hearts clamped after 15 min working heart perfusion.

Results are Mean ± SEM (number of measurements).

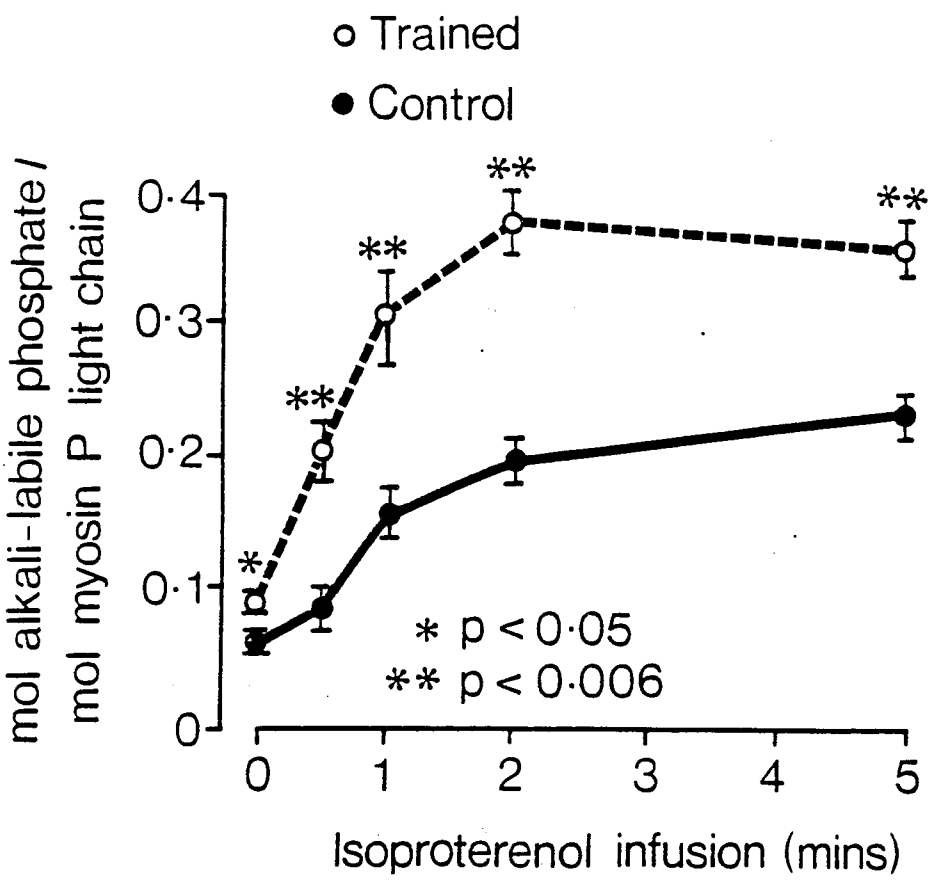

Figure 6.16

Incorporation of alkali-labile phosphate into myosin light chains of hearts from trained and control rats after varying periods of isoproterenol infusion.
Note that phosphate incorporation is significantly higher in trained hearts.
Data of Resink and Gevers, published with permission.

(data not shown). When data from all the different perfusate calcium concentrations were studied, there was a significant linear correlation between the maximum velocities of myosin Ca^{++}-ATPase activities and levels of myosin P light chain phosphorylation (Fig. 6.17).

Myocardial cyclic AMP levels.

Myocardial cyclic AMP levels at varying times after isoproterenol infusion at 2 different perfusate calcium concentrations are listed in Table 6.7. There were no differences in these levels between trained and control hearts at any time, or at either perfusate calcium concentration.

6.6 DISCUSSIONS AND CONCLUSIONS.

There are 4 principal findings in these studies.

A. Steady state vs. changing haemodynamic conditions.

First, these studies show that the choice of the perfusion protocol used to study the myocardial adaptations to exercise training may influence the results obtained. When hearts were perfused under conditions of changing atrial filling pressures and heart rates, with isovolumic beats interspersed, there were differences in left ventricular function (Fig. 6.8) that were absent under steady-state perfusion conditions (Figs. 6.1 - 6.7; 6.10; 6.12 - 6.14).

This observation has not previously been made although it should be noted that hearts from swimming-trained rats which have enhanced mechanical function when studies in the isolated perfused system[209,251,252,260-262] under conditions of changing atrial filling pressures, do not show these adaptations when studied under more constant conditions *in vivo*[210,318].

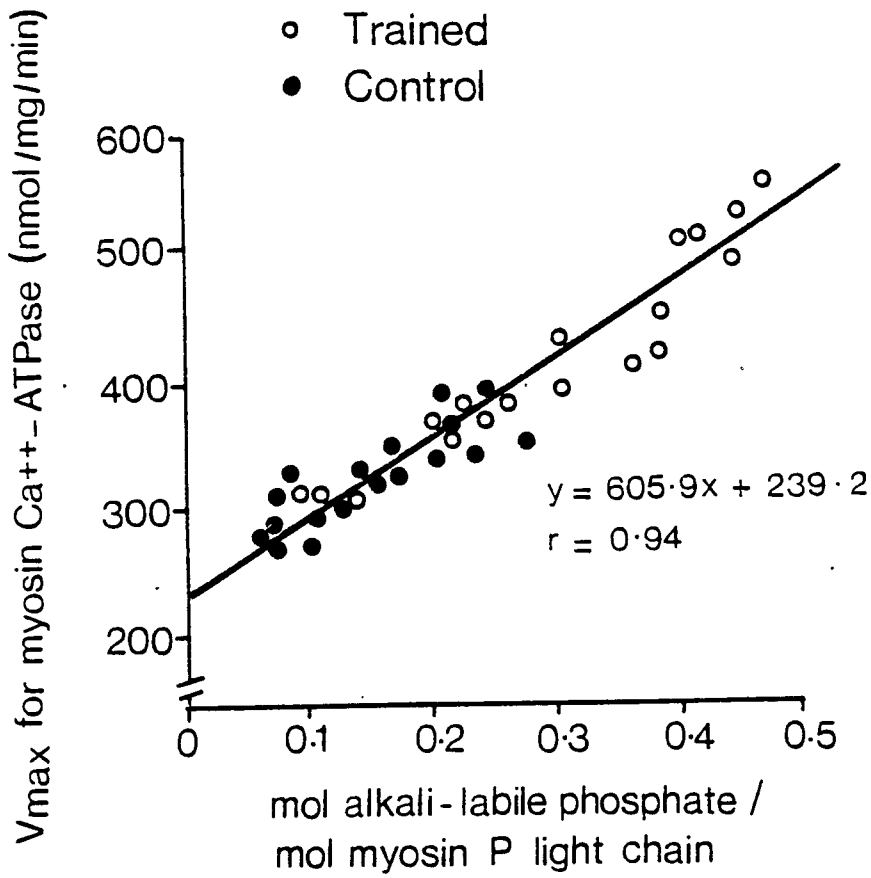

Figure 6·17

A significant linear correlation between the V_{max} values for myosin Ca^{++}-ATPase activities and the phosphorylation levels of myosin P light chains was found when data from all experiments with trained and control animals were included.
Data of Resink and Gevers, published with permission.

In accord with the work of Schaible & Scheuer[262], under conditions of changing atrial filling pressures, stroke volumes were significantly higher in running-trained than in control hearts without there being significant differences in either L.V. max +ve or max -ve dP/dt values. Rates of coronary flow and myocardial oxygen consumption were also significantly higher in hearts from trained rats in this study, but not in that of Schaible and Scheuer. This difference might be explained by the less severe perfusion conditions used by Schaible and Scheuer.

The reason why a protocol using changing workloads should be necessary to demonstrate myocardial adaptations to training is not known, but must presumably be related to the mechanism whereby catecholamine stimulation also brings out these differences (sections 6.6C and 6.6D).

B. Effect on training on myocardial glycolytic rates.

Second, these studies have established that glycolytic rates are significantly higher in hearts from trained rats perfused with glucose/insulin under steady-state conditions both before and after β-stimulation with isoproterenol (Fig. 6.11).

Kobayashi and Neely[440] have shown that maximum glycolytic rates of isolated working rat hearts perfused aerobically with glucose/insulin may be limited by the accumulation of cytoplasmic NADH which inhibits the enzyme, glyceraldehyde-3-P-dehydrogenase. Thus a possible explanation for the increased glycolytic rates measured in trained hearts may be increased capacities for hydrogen transport into the mitochondria, as has been found in trained skeletal muscles[484]. The lower rates of lactate release from trained hearts perfused with glucose/insulin (Table 6.3) provide supportive evidence for this interpretation, because lactate release is an important alternative mechanism for cytoplasmic NAD regener-

ation from $NADH^+$, when the rate of glycolytic $NADH^+$ production exceeds hydrogen ion transport capacity into the mitochondria via either the malate/aspartate or the α-glycerophosphate shuttles. Although Kraus and Kirsten[185] found mitochondrial α-glycerophosphate dehydrogenase to be increased in hearts from swimming trained rats, others have failed to find this adaptation[171]. Increased concentrations of glycolytic enzymes including LDH and pyruvate kinase have been reported in trained hearts (section 2.3B), but there have been no previous reports directly measuring glycolytic rates in hearts from trained and control animals in vitro.

The greater glycolytic capacity of trained hearts should be beneficial under conditions of impaired myocardial oxygenation. Isolated, perfused working hearts from swimming-trained rats have been shown to exhibit superior cardiac performance during both hypoxia[252] and ischaemia[251]. Only during ischaemia were rates of lactate release greater in hearts from swimming-trained rats. Hearts from running-trained rats also had superior function measured in vivo during hypoxia,but not during acute regional ischaemia[152]. In that study no biochemical explanation was found for the superior myocardial function of trained hearts during hypoxia, but glycolytic rates were not measured.

The finding that glycolytic rates calculated from substrate uptake data, were not significantly greater in trained hearts suggests that this indirect method may be too insensitive to identify small, training-induced changes in myocardial metabolism. The only previous study that has shown training-induced changes in the metabolism of isolated perfused hearts also used radioactive techniques[250].

From the arguments developed in the previous chapter, it would have been expected that the higher rates of myocardial glycolysis measured

in trained hearts during control perfusions (Fig. 6.11) would have been associated with greater rates of myocardial relaxation and higher stroke volumes under those conditions. It is possible that the measured differences in the rates of myocardial glycolysis were too small to influence myocardial function during fully-oxygenated perfusions.

C. Effects of β-stimulation with isoproterenol at different perfusate $CaCl_2$ concentrations.

Third, these studies show that even under steady-state perfusion conditions, differences in myocardial function between trained and control hearts can be elicited by β-stimulation (Figures 6.10 and 6.13). These functional differences (increased stroke volumes, increased peak left ventricular systolic pressures, increased LV max +ve and max -ve dP/dt values) were not associated with an altered chronotropic response to β-stimulation.

Others have shown that β-agonists exaggerate differences in function between hearts from trained and control hearts. Wyatt, Chuck, Rabinowitz et al[244] and Mole[259] found that isoproterenol infusions magnified differences in rates of tension development by isolated papillary muscles from trained and control animals. In chronically-instrumented unanaesthetized dogs, Stone[239] reported that, in response to isoproterenol infusions, trained dogs had greater cardiac outputs, stroke volumes, circumflex coronary artery flows and greater increases in maximum rates of left ventricular pressure development than had been measured in the untrained state. In contrast, Nutter and Fuller[256] and Williams and Potter[257] did not find any difference in the mechanical response of isolated papillary muscles from trained and control animals to norepinephrine infusions. In open-chested rats, although isometric tension

development was greater in hearts from swimming-trained animals under control conditions, during adrenaline or isoprenaline infusions control hearts developed greater tension[317]. The findings that the heart rate response to isoproterenol infusions was not different between hearts from trained and control animals is in accord with findings in isolated right atria[220] and in intact dogs[239] but at variance with findings in isolated Langendorff-perfused rat hearts[229] and intact rats[229,384,387], in all of which studies significantly greater heart rate responses were measured in trained animals.

The differences in mechanical function between trained and control hearts after isoproterenol infusion were not associated with different tissue cyclic AMP levels (Table 6.7). It should also be noted that whereas cardiac function increases progressively in both trained and control hearts with increasing perfusate calcium concentrations (Figures 6.12-6.14), tissue cyclic AMP levels were not different between any groups of hearts when measured at the 2 lower perfusate $CaCl_2$ concentrations (Table 6.7). No previous studies have reported the effects of exercise training on myocardial cyclic AMP responses to β-stimulation. However, Dohm, Pennington and Barakat[243] reported that basal- and epinephrine-stimulated myocardial adenylcyclase activities were reduced in hearts from running-trained rats whilst myocardial phosphodiesterase activities were unchanged. In contrast, Wyatt, Chuck, Rabinowitz et al[244] found that both isoprenaline and norepinephrine caused significantly greater adenyl-cyclase activities in hearts from swimming-trained cats.

Probably the most critical observation in this chapter is that the enhanced response of trained hearts to β-stimulation is lost when the $CaCl_2$ concentration is increased to unphysiological levels (Fig. 6.14). This indicates that the differences in mechanical function between hearts from

trained and control rats after β-stimulation at physiological perfusate Ca^{++} levels must be calcium-mediated and are presumably due to an enhanced capacity for trans-sarcolemmal Ca^{++} flux in trained hearts. The additional biochemical evidence for this conclusion is discussed below.

Although mechanical data were not collected at the lowest $CaCl_2$ concentration (1,6 mM) after β-stimulation, one would expect the differences in mechanical function between trained and control hearts to be magnified at low perfusate calcium concentrations because, under such conditions, the greater trans-sarcolemmal calcium transport capacity of the trained hearts would insure greater calcium transport to the contractile proteins. In this context it should be noted that Scheuer and his colleagues have consistently used perfusate calcium concentrations below 2,2 mM in their studies of heart function in trained and control rats.

A further point of interest was the finding that, in control hearts during β-stimulation, the rates of myocardial relaxation increased with increasing perfusate calcium concentrations (compare Figures 6.13 and 6.14). If one assumes that the higher level of myocardial function measured at the higher perfusate calcium concentrations (in both trained and control hearts) is due to increased levels of intracellular calcium during contraction, then this finding suggests that the rate of calcium uptake by the sarcoplasmic reticulum may, in part, be controlled by the level to which intracellular calcium rises during contraction. Thus one might propose that the higher rates of myocardial relaxation of trained hearts might reflect their greater capacity for calcium transport and the resulting higher intracellular calcium concentration. Thus running training may influence sarcoplasmic reticulum function only in so far as it alters intracellular calcium concentrations during myocardial contraction.

This interpretation would be in accord with the finding that isolated sarcoplasmic reticulum from running-trained animals do not show increased rates of calcium uptake in vitro (section 2.3D) but would not exclude the possibility that, in vivo, trained hearts could still have greater rates of left ventricular relaxation.

D. Myosin and actomyosin ATPase activities, troponin I and myosin P light chain phosphorylation.

Fourth, in agreement with others,these studies show that rat myocardial actomyosin ATPase activities (Table 6.5) are not altered by treadmill running training (section 2.3D). However, in contrast to others[174,215,216], we found that myosin Ca^{++}-ATPase activities were significantly higher in hearts from running trained rats (Tables 6.5, 6.6 and Fig. 6.15), that the increased myosin Ca^{++}-ATP ase activities were associated with increased myosin P light chain phosphorylation levels (Fig. 6.16) and that there was a significant correlation between these 2 parameters (Fig. 6.17). Thus one explanation why other workers have not found increased myosin Ca^{++}-ATPase activities in running-trained hearts would be that they failed to maintain in situ states of myofibrillar protein phosphorylation because they did not include potent inhibitors of endogenous phosphoprotein phosphatase(s) during their isolation and purification procedures. On the basis of these findings, it is suggested that the increased capacity for nett phosphorylation of myosin P light chains explains the increased myosin ATPase activities of trained hearts[484].

Myosin P light chain phosphorylation is brought about by the action of the enzyme myosin light chain kinase, which is a Ca^{++}-dependent enzyme, and this ion dependence is a function of its calmodulin subunit[485,486]. Increased phosphorylation rates after training could therefore occur in several ways[484]:

(i) Modification of myosin P light chain susceptibility to phospho-

rylation by myosin light chain kinase.

(ii) Enhanced levels of myosin light chain kinase in hearts of trained animals.

(iii) Decreased capacity or function of myosin light chain phosphatase.

(iv) Increased availability of Ca^{++} to the light chain kinase system either through enhanced influx through the slow calcium current or via a decrease in the Ca^{++}-sequestering activity of the sarcoplasmic reticulum.

Of these possibilities, no measurements of myosin light chain kinase and phosphatase activities have yet been reported in studies of hearts from trained animals, and there is also no evidence to suspect that trained hearts have a reduced capacity for Ca^{++} accumulation by the sarcoplasmic reticulum (section 2.3D and Fig. 6.13). On the other hand, our mechanical data (see above) together with that of Tibbits and his colleagues [216,392] provide strong evidence for enhanced Ca^{++} transport from the extracellular space to the contractile proteins, and therefore to the light chain kinase system.

It is therefore postulated that an enhanced trans-sarcolemmal Ca^{++} transport resulting from exercise training, explains both the increased mechanical performance of trained hearts and their greater levels of myosin P light chain phosphorylation and higher Ca^{++}-ATPase activities[487]. The finding that the differences in left ventricular function between trained and control hearts were least (Fig. 6.14) when the trained hearts reached their highest Ca^{++}-ATPase activities (Table 6.6) and myosin P light chain phosphorylation levels (data not shown) indicates that, in these experiments, these 2 parameters were probably not the principal determinants of the differences in left ventricular function between trained and control hearts.

Rather, it would seem that they reflect the higher intracellular

calcium concentrations reached by the trained hearts. This further suggests that, above a certain level of intracellular calcium (and therefore a certain level of myosin P light chain phosphorylation and Ca^{++}-ATPase activity), no further increase in left ventricular function will occur.

The final question that needs to be discussed relates to the role of cyclic AMP in mediating these differences, because increased extracellular calcium concentrations can have an activating effect on both adenylcyclase[488,489] and on the calmodulin-cyclic nucleotide phosphodiesterase inhibitor[489] such that myocardial cyclic AMP levels are increased. However, it was found that increasing the extracellular $CaCl_2$ concentration from 1,6 to 2,2 mM had not effect on either myocardial cyclic AMP levels nor on the cyclic AMP response to isoproterenol infusion, which was in any case not different between trained and control hearts (Table 6.7).

These findings therefore suggest that a cyclic AMP-linked mechanism is not responsible for either the biochemical or mechanical differences observed between hearts from trained and control rats, either in the presence or absence of catecholamines, and that these differences result from an increased capacity for trans-sarcolemmal calcium transport resulting from running training.

CHAPTER 7.

THESIS SUMMARY AND CONCLUSIONS.

DIRECTIONS FOR FUTURE RESEARCH.

"The world is round and the place which may seem like the end may also be only the beginning".

IVY BAKER PRIEST (1958).

7.1 INTRODUCTION

Each chapter of this thesis has concluded with a summary of its most relevant findings. In this concluding chapter, the principal findings of each chapter will be briefly restated and their relevance to future research described.

7.2 Coronary heart disease in marathon runners.

The finding that coronary atherosclerosis and sudden cardiac death can exist in marathon runners indicates that exercise alone is not the complete answer to coronary heart disease. Furthermore, the fact that some runners developed this disease in the absence of any of the classic coronary risk factors confirms that much is still unknown about the factors predisposing to this disease. The paper by Marmot, Rose, Shipley and Hamilton[27] is especially relevant to this question because it indicates the strong influence social class and employment grade have on coronary mortality and emphasizes that only 40% of the difference in mortality between those with low, and those with high, coronary mortality rates can be explained on the basis of the "traditional" coronary risk factors, namely blood pressure, serum cholesterol, smoking, and height.

Although it is unlikely that further studies of marathon runners will necessarily uncover these unknown coronary risk factors, it should be noted that, as a group, marathon runners should have a low incidence of the traditional coronary risk factors. Thus most marathon runners are of higher social class[490] and very few smoke[490,491]. Furthermore they should receive additional protection from this disease because of higher HDL-cholesterol levels[492,493], lower fasting insulin levels and superior glucose tolerance[494]. Thus, it is possible that these others,

as yet unknown, coronary risk factors might be more easily recognized in the marathon population, because of their lower incidence of the classic major, risk factors.

The findings of this study have bearing on three important questions:

1) The role of exercise in preventing the progression of established coronary atherosclerosis. It is clear from some cases described in this thesis, that a "malignant" form of coronary atherosclerosis exists, in which there is rapid disease progression despite correction of all known coronary risk factors. In persons with such a severe form of the disease, exercise is unlikely to be of value. On the other hand, exercise training might reduce the rate of disease progression in persons with a less severe form of the disease, as suggested by the initial studies of Selvester, Camp and Sanmarcos[69]. There is clearly a need for further studies using the same experimental design as that of Selvester and his colleagues.

2) The opposite hypothesis, namely that in persons with advanced coronary atherosclerosis severe exercise like marathon running might increase the rate of disease progression, or alternatively produce myocardial injury that hastens the course of the disease, also needs to be considered. This unpleasant possibility is suggested by the finding that marathon running had an apparently detrimental effect on some of the runners reported in this study. It should be noted that a recent paper has reported that the longevity of rats with genetic hypertension and obesity was reduced by daily running[495]. Exercised, diseased rats also had an increased incidence of focal myocardial necrosis.

3) A question which has not been adequately studied is why the myocardial potassium to sodium ratios are low in cases of sudden cardiac death, as found in the hearts of 2 of the runners described in this study. In experimental coronary artery occlusion, myocardial K^+/Na^+ ratios fall gradually, reaching values below 1,2, only 130 minutes after the onset of myocardial ischaemia[496]. As far as I am aware no one has yet considered the possibility that low myocardial K^+/Na^+ ratios may be the cause, rather than the result,of ventricular fibrillation as is currently believed[402].

Finally, these studies show the need for continuing education of medical practitioners, so that they might better appreciate the importance of adequate cardiac evaluation of runners with symptoms and,of runners, so that they might realize that warning symptoms need early, comprehensive, medical evaluation. The need for the better autopsy evaluation of all cases of sudden death in athletes has also been emphasized.

7.3 Effects of exercise training on ventricular fibrillation thresholds.

The encouraging results that exercise training increased ventricular fibrillation thresholds in rats, should stimulate further interest in this potentially-important protective effect of training. For example, it is possible that the reduction in the sudden death rate in post-myocardial infarction patients participating in intervention programmes which include exercise training[497,498], may be due to increased myocardial resistance to lethal arrhythmias. Furthermore, this adaption may not be associated with changes in any of the conventional risk factors, nor with changes in either coronary flow patterns or left ventricular function, changes which have, in any case, not been convincingly demonstrated in trained cardiac patients (section 2.3F).

There is an urgent need for future research to identify the serious warning arrhythmias and to determine whether exercise training reduces their frequency. This information is likely to be more rewarding than will be further studies of the myocardial functional adaptations in trained cardiac patients. Alternatively, if the biochemical basis for ventricular fibrillation were to be discovered, a beneficial effect of exercise training might be assumed if exercise were shown to beneficially alter this factor or factors.

In passing, it is of interest that calcium may be an important factor in the genesis of ventricular arrhythmias[499]. Thus the evidence that exercise training increases the rates of myocardial calcium transport (Chapter 6) would predict increased susceptibility to ventricular fibrillation in trained hearts. Clearly another factor is protecting trained hearts during those situations in which an increased calcium transport capacity may be deleterious.

7.4 Maximum work in the isolated perfused rat heart model.

The principal finding for future research was that glycolytically-produced ATP may be an important source of energy for myocardial relaxation. To confirm that this relationship is causal, it will be necessary to show that isolated sarcoplasmic reticulum has the capacity to generate ATP from glucose, and that such ATP can be used for calcium uptake.

This relationship is of interest to the exercise situation, because it begs the question whether during prolonged exercise, myocardial glycogen levels can be depleted (as occurs in skeletal muscle) to the point where myocardial glycolytic rates are critically reduced and myocardial compliance impaired. During the course of this thesis, 4 cases

of acute pulmonary oedema were uncovered in 2 competitors in the 1978 Comrades Marathon[500]. It was postulated that reduced myocardial compliance occurring only during exercise which was sufficiently prolonged to reduce rates of myocardial glycolysis, could have explained why these athletes developed pulmonary oedema only when they ran for more than 4-5 hours.

It is of particular interest that the myocardium resyntheses glycogen much more rapidly after exercise than does skeletal muscle[163], and that such resynthesis occurs even during fasting[501]. It would be illogical to think that this difference is not teleologically-significant. Possibly it results from the need to insure that myocardial glycolytic rates are always maintained at levels that insure adequate myocardial compliance.

Finally, the finding that the extracellular calcium concentration influences the rate of myocardial relaxation needs to be studied further, as does the finding of substrate-induced differences in myocardial efficiency. If myocardial glycolysis is indeed involved in the energetics of myocardial relaxation, on this basis, glycolytic rates should be responsive to intracellular calcium levels. The finding that the addition of glucose-insulin to lactate- or palmitate-perfused hearts improves myocardial efficiency may provide a lead to the factor(s) determining substrate-induced differences in myocardial efficiency.

7.5 Myocardial adaptations to training.

The increased rates of myocardial glycolysis measured in trained hearts should be beneficial under conditions of either myocardial ischaemia or hypoxia. Although swimming-trained hearts have been shown to perform better under conditions of ischaemia and hypoxia, similar studies have not

been performed in running-trained animals nor has the role of myocardial glycolysis in explaining the superior function of swimming-trained rats under these conditions been defined.

The major finding of this study was that trans-sarcolemmal calcium transport capacity seems to be increased in trained hearts and that this adaptation leads to increased levels of myosin P light chain phosphorylation. On this basis it is postulated that increased calcium transport capacity is the principal factor explaining the superior myocardial performance of hearts from trained animals. It is therefore of interest that,during exercise, serum ionized calcium levels increase[502]. If physical training were also to increase the levels to which serum ionized calcium rises during exercise, then both adaptations would provide a synchronized, complementary method for increasing myocardial performance during exercise. This adaptation would then insure that the training-induced reduction in sympathetic tone during exercise[233-236] does not also reduce myocardial performance capacity.

With regard to training-induced adaptations in myocardial metabolism, there is a need for further studies using radioactive techniques in the isolated perfused working rat heart model, and for additional studies in humans.

Finally, future studies should explore the possibility that running- and swimming-training in rats produce different myocardial adaptations. Differences that have been described are heart weights after training (section 2.3A), myosin and actomyosin ATPase activities (section 2.3B), calcium uptake by isolated sarcoplasmic reticulum (section 2.3C) and the activities of myocardial adenyl cyclase and phosphodiesterase (section 2.3B). Swimming- and running- training represent quite different psychological

stresses for rats, but they also produce quite different blood flow responses to either exercise[503]. Ultimately, one would like to know which exercise type reproduces those myocardial adaptations that are likely to be found in trained humans.

APPENDIX 1

Methodology of studies in the isolated perfused rat heart model

APPENDIX 1

I. METHODOLOGY OF STUDIES IN THE ISOLATED PERFUSED RAT HEART MODEL.

A. The rat exercise training programme.

Male inbred Wistar-Weissman or Long-Evans rats with an initial body weight of 140 grams were randomly assigned to either a sedentary or training group. The training programme lasted for a minimum of 8 weeks. The animals exercised 5 days a week on a Quinton Model 42 - 15 treadmill (Quinton Instruments, Washington) set at a 15 degree incline. On the first and second training days, the rats ran for 15 minutes, on the third day for 30 minutes, on the fourth day for 45 minutes and on the fifth day for 60 minutes. Thereafter the running time was increased by 3 minutes daily until, at the end of the fifth training week, the rats were running for 2 hours daily - the first hour at up to 0,8 miles per hour, the second at up to 1 mile per hour. This exercise level was maintained until the rats were sacrificed beginning after 8 weeks' training. Untrained rats were kept at normal cage activity. All rats were exposed to a 12 hour day/night cycle and were fed with rat cubes and water ad libitum.

B. The perfusion fluid.

The perfusion fluid used in all experiments was a modified Krebs-Henseleit[504] buffer gassed with 95% O_2:5% CO_2 at a temperature of 37^{o}C. The Krebs-Henseleit buffer was made up from the following stock solutions, added in the volumes indicated, to make up 5 litre solutions:

NaCl	138,46 g/l	250 ml/5l
$NaHCO_3$	42 g/l	250 ml/5l
KCl	35.4 g/l	50 ml/5l
KH_2PO_4	16,13 g/l	50 ml/5l
$MgSO_4$	29,32 g/l	50 ml/5l
$CaCl_2.2H_2O$	36,8 g/l	50 ml/5l

The Krebs-Henseleit buffer was prepared from ice-cold solutions which were gassed with 95% O_2:5% CO_2 for 20 min before the $CaCl_2$ was added. This was necessary to lower the solution pH below that at which calcium precipitation occurs.

A variety of substrates were added to the perfusion fluid either singly or in combination. These substrates were:

11,1 mM D(+) glucose (Merck, Darmstadt, West Germany).

10 mM DL β-OH butyric acid (butyrate) (Sigma Chemical Company, St. Louis, Missouri).

10 mM L(+) lactic acid (lactate) (ICN Pharmaceuticals, Cleveland, Ohio).

10 mM pyruvic acid (pyruvate)(Boehringer, Mannheim, West Germany).

1 mM palmitic acid (palmitate)(Sigma Chemical Company) bound to 3% albumin (Bovine Albumin Fraction V, Sigma Chemical Company)

In some experiments, insulin (NUSO Neutral Insulin$^{(R)}$, Wellcome Foundation) at a concentration of 2 u/litre or isoproterenol (Isuprel$^{(R)}$ - Winthrop Laboratories) at a concentration of $6,5 \times 10^{-7}$M were used. When radioactively labelled ^{3}H-glucose (D-[2-^{3}H(N)] - glucose. New England Nuclear, Boston, Massachusetts) was added to the perfusate (see below), the ethanol in which the ^{3}H-glucose is stored, was first evaporated off by carefully directing a jet of compressed air (Medical Air, African Oxygen Limited) into a centrifuge tube (Eppendorff)

containing the appropriate volume of ^{3}H-glucose solution (100 µl/litre Krebs-Henseleit buffer).

C. Perfusion fluid analyses.

Immediately on termination of those experiments measuring myocardial metabolosm (sections 5.2B and 6.2), perfusion fluid aliquots were deep frozen pending analysis by the following methods:

D(+)-glucose (Method of Bergmeyer, Bernt, Schmidt et al[505]).

Glucose was assayed by an NADP coupled system based on the following reaction:-

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{G6P} + \text{ADP}$$

$$\text{G6P} + \text{NADP}^{+} \xrightarrow{\text{G6PDH}} \text{6 phosphogluconate} + \text{NADPH} + \text{H}^{+}$$

The amount of glucose present is proportional to the formation of NADPH as measured by the change in extinction at 340 nm brought about by the addition of 10 µl of equal quantities of hexokinase (10 mg/ml) and glucose-6-phosphate dehydrogenase (G6PDH)(1 mg/ml) to the following reaction mixture:

Tris buffer pH 7,5 0,2M	1,0 ml
NADP 1%	0,1 ml
ATP 20 mM	0,1 ml
$MgCl_2$ 1M	0,1 ml
H_2O	1,7 ml
sample	200 µl

All glucose analyses were completed within 18 hours of completion of the experiments.

L(+)-lactate (Method of Gutmann and Wahlefeld[505])

Samples for the determination of lactate were de-proteinized immediately upon collection with 6% perchloric acid in the ratio of 0,2 ml 6% perchloric acid to 1 ml of perfusion fluid.

The measurement of lactate was based on the following reaction:

$$\text{L(+) lactate} + NAD^+ \xleftrightarrow{LDH} \text{Pyruvate} + NADH + H^+$$

The amount of L(+) lactate present is proportional to the formation of NADH which is measured by the change in extinction at 340 nm brought about by the addition of 5 µl lactate dehydrogenase (LDH 10 mg/ml) to the following reaction mixture:

Buffer (hydrazine-glycine-EDTA-NaOH) pH 9,5	1,5 ml
NAD 1%	0,2 ml
H_2O	1,1 ml
Sample	0,2 ml

Pyruvate (Method of Czok and Lamprecht[507])

$$\text{Pyruvate} + NADH + H^+ \xleftrightarrow{LDH} \text{lactate} + NAD^+$$

The oxidation of NADH is proportional to the amount of pyruvate converted and is measured by the change in extinction at 340 nm brought about by the addition of 5 µl lactate dehydrogenase (LDH 10 mg/ml) to the following reaction mixture:

Tris buffer pH 7,5 0,2M	0,9 ml
NADH 0,4%	0,1 ml
Sample	2,0 ml

Free fatty acids

Perfusate free fatty acid concentrations were determined by a titrimetric procedure devised by Chlouverakis[508] and based on the method of Dole[509] . Following extraction of 0,6 ml of perfusate with 3 ml of Dole's extraction mixture (isopropyl alcohol 40 parts, heptane 10 parts, 1N H_2SO_4 1 part) the system was divided into two phases by the addition of 1,8 ml of heptane and 1,2 ml of water. At least 10 minutes after the two phases had separated,1 ml of the upper phase was pipetted into conical tubes to which 1 ml of nile blue indicator (0,02% weight for volume in 90% volume for volume ethanol) was added. Titration was then carried out against standardised 0,01N NaOH with an Aglar micrometer syringe burette under a stream of nitrogen to prevent CO_2 adsorption and to ensure proper mixing.

^{3}H-sorbitol

To calculate the volume of fluid circulating during those experiments studying myocardial metabolism, 50 µl of a solution of ^{3}H-sorbitol (Amersham, Buckinghamshire) diluted 1:1 in distilled water, was added to the perfusion fluid. At the end of the experiments, 200 µl aliquots from the perfusate samples collected both at the beginning (before the addition of ^{3}H-sorbitol to the perfusate) and at the end of the experiments (after ^{3}H-sorbitol addition) were each diluted in 10 ml of scintillation fluid (Insta-Gel, Packard Instrument Company, Downers Grove, Illinois) thoroughly mixed, wiped clean and counted in a Beckman Liquid Scintillation Counter (Beckman Instruments, Fullerton, California).

Coronary effluent $^{3}H_2O$.

Measurement of $^{3}H_2O$ in the coronary effluent in hearts perfused with D-2-^{3}H glucose allowed calculation of glycolytic rates according to the methods of Neely, Denton, England and Randle[439].

Ion exchange resin (Resin Dowex 1 x 4-200 - Sigma Chemical Company) was first prepared according to instructions kindly sent to us by Professor M.J. Rovetto of the Department of Physiology, Jefferson Medical College of the Thomas Jefferson University, Philadelphia.

After preparation, the resin was placed over glass wool in Pasteur pipettes to a column height of 2,5 cm. The resin was thoroughly rinsed with distilled water before being dried with compressed air (Medical Air, African Oxygen Limited). An 0,5 ml aliquot of coronary effluent was then carefully washed through the resin with 3 volumes of distilled water, 0,5 ml per passage into 10 ml of scintillation fluid (Insta-Gel, Packard Instrument Company).

Standards and blanks were both prepared from aliquots of perfusate that had not passed through the heart. Standards were prepared by adding 0,5 ml of perfusate plus 1,5 ml of distilled water directly to the scintillation fluid without passage through the column, whereas blanks were prepared by the passage of the perfusate aliquot through the resin columns in the same manner as that used in the preparation of the coronary effluent samples.

After the perfusate or coronary effluent had been washed through the column with distilled water, the resin columns were again dried with the passage of compressed air. The scintillation vials were then spun to ensure complete mixing, wiped clean, and their radioactivity determined by counting in a Beckman Liquid Scintillation Counter.

Oxygen tension.

Oxygen tensions in the perfusate and coronary effluent samples were measured by a Radiometer macroelectrode (Type PHMM1; Radiometer, Copenhagen). The perfusate was drawn under vacuum into a 1 ml syringe, either from the pulmonary artery (corresponding to the venous oxygen tension - P_vO_2) or from a sidearm positioned on the left atrial cannula, this value representing the arterial oxygen tension - P_aO_2). All samples were analyzed immediately they had been taken.

Perfusate free calcium.

Perfusate free calcium concentrations were measured using a prototype calcium sensitive electrode made by Dr. J.G. Schindler (Marburg, West Germany) and tested in South Africa by Dr. H. Maier (Department of Biomedical Engineering, University of Cape Town).

D. Tissue biochemical analyses.

At the termination of the experiments, perfused hearts were treated in either of two ways.

1) Hearts were rapidly removed from the perfusion cannulae, the atria excised and the right and left ventricles separated, blotted dry and weighed separately. A section of each left ventricular apex was then removed, immersed in liquid nitrogen and stored for the later analysis of glycogen contents. The remaining portions of the left ventricles, and the right ventricles, were then incubated separately at 110°C for 5 days before re-weighing for the calculation of dry and fresh heart weights.

2) In all other experiments, hearts were clamped, as described in the text, with Wollenberger tongs precooled in liquid nitrogen. The

atria together with the excess fluid at the edge of the hearts were then chipped off, and the hearts placed in individually-labelled plastic bags, for storage in liquid nitrogen pending later biochemical analysis.

For biochemical analyses, frozen hearts were treated in the following way. The stored tissue samples were powdered in a metal percussion mortar maintained at the temperature of liquid nitrogen until a powder sufficiently fine for extraction, was produced.

Dry heart weights.

The first aliquot of about 200 mg frozen tissue was transferred to a pre-weighed empty glass container and allowed to thaw. The glass tube was then re-weighed immediately, care being taken to remove any moisture that might have accumulated on the outside of the glass tube. These tissue samples were then dried at 110°C until constant dry weights were obtained.

Tissue glycogen.

The second aliquot of 60-70 mg tissue was transferred to a pre-weighed centrifuge tube (Eppendorff, Hamburg, West Germany) containing 0,2 ml of cold 40% potassium hydroxide for the determination of glycogen by a modification of the method of Good, Kramer and Somogyi[510]. The tube and tissue were then re-weighed and the tissue weight obtained.

The tube was next heated to 95°C on an Eppendorff thermostat for 30 minutes being shaken every 10 minutes. Thereafter 0,8 ml absolute alcohol was added and the tube refrigerated overnight.

On the following day, the tube was spun for 2 minutes in an Eppendorff centrifuge. The supernatant fluid was then aspirated and the remaining pellet washed at least twice with cold absolute alcohol, and

the supernatant fluid aspirated each time. 0,2 ml of 2N HCl was next added to the pellet and the tube heated for 3 hours on the Eppendorff thermostat at 95°C, after which the fluid was neutralized to a pH between 7,5 and 7,7 using 5 µl of Universal Indicator, and added to a mixture containing the following:

0,2 M pH 7,6 Tris buffer	1 ml
10 mM ATP	0,2 ml
1M $MgCl_2$	0,1 ml
1% NADP	0,1 ml
H_2O	1,2 ml

The fluid was then assayed according to the same method used for perfusate glucose measurements (Appendix 1.C). 1 mM glucose standards and blanks were also assayed.

ATP, PCr, citrate, lactate, cyclic AMP and cyclic GMP.

A third aliquot of about 300 mg was placed in a pre-weighed tube containing 1,2 ml ice-cold 5% PCA. After re-weighing the tube to calculate the weight of the added tissue, the contents of the tube were thoroughly mixed by homogenizing/sonicating with a Polytron homogenizer (Lucerne, Switzerland). Care was taken throughout the procedure to keep the extraction mixture ice-cold.

The homogenate was then centrifuged for 10 minutes at 5000 rpm and at 4°C in a Beckman Model J-21 centrifuge. 1 ml of the supernatant fluid was taken off, pipetted into an Eppendorff tube and neutralized, as judged by a universal indicator (5 µl), to pH 7,0 with a Tris/KOH/KCl solution consisting of 0,2 M Tris, pH 7,5 and 40% KOH saturated with KCl. The Tris and KOH/KCl solutions were mixed in the volume ratio of 6:4 respectively. The volume of added Tris/KOH/KCl was noted, and the

solution allowed to precipitate for 30 minutes, after which it was re-centrifuged in the Eppendorff centrifuge, and the resulting supernatant decanted in fresh Eppendorff containers and stored at $0^{o}C$ until assayed for the following intermediates:

ATP (Method of Lamprecht and Trautschold[511])

$$\text{ATP + glucose} \xrightarrow{\text{hexokinase}} \text{G6P + ADP}$$

$$\text{G6P + NADP}^{+} \xrightarrow{\text{G6PDH}} \text{6-Phosphoglucono-d-lactone}$$

ATP was assayed by the change in extinction at 340 nm on the addition of hexokinase (5 µl of 10 mg/ml) to a mixture of 0,2 ml of heart extract and 2,8 ml of assay solution. The assay solution contained the following:

$MgCL_2$ 1M	0,1 ml
Tris buffer 0,2M pH 7,5	1,0 ml
NADP 1% w/v	0,1 ml
Glucose 100 mM	0,05 ml
H_2O distilled	1,55 ml
G6PDH 1 mg/ml	5 µl

When the change in extinction had stabilised, 0,05 ml 10 mM ADP was added to provide excess ADP for the subsequent assay of phosphocreatine, and any further change in extinction was noted.

PCr.

$$\text{Phosphocreatine + ADP} \xrightarrow{\text{creatine kinase}} \text{ATP + creatine}$$

Phosphocreatine was then assayed by the further change in extinction at 340 nm on addition of 10 µl creatine kinase (10 mg/ml made up in distilled H_2O) to the reaction mixture in which the ATP assay had been completed. 20 to 30 minutes were allowed for this reaction

to go to completion.

Standards and blanks were run with each assay.

Citrate.

Citrate was measured using the fluorometric method of Passonneau and Brown[512].

The reaction mixture was made up of:

2,5 ml	1M Tris buffer pH 8
50 µl	5 mM $NADH_2$-Na_2
100 µl	1 M MgCl
50 µl	0,1M Ethylenediaminetetra-acetate (EDTA)
100 µl	MDH (0,2 mg/ml)

made up to 50 ml with distilled water.

10 µl of sample was added to 1 ml of the reaction mixture and the difference in extinction obtained when 10 µl of citrate lyase was added and read after 30 minutes. A 1 mM standard was used, and distilled water was used as the blank.

Lactate.

The assay is the same as that for perfusate lactate (described previously).

A 10 µl sample was added to 1 ml cocktail and the change in extinction at 340 nm was recorded 30 minutes after the addition of 5 µl LDH.

Cyclic AMP. (Method of Torey, Oldham and Whelan[513]).

These assays were kindly performed by Miss Cecile Muller, Research Fellow in the Heart Unit, who supplied the following assay description.

The assay is based on competition between unlabelled cAMP in the tissue sample and a fixed quantity of tritium-labelled cAMP, for binding to a bovine protein which has a high specificity and affinity for the nuclide. The quantity of labelled protein-cAMP complex formed is inversely proportional to the amount of cAMP present in the tissue sample. Measurement of the protein-bound radioactivity enables the amount of unlabelled cAMP to be calculated. A 50 µl aliquot of the tissue sample together with 50 µl of the radioactive cAMP is incubated with the protein for 18 hours. Activated charcoal is then added to remove the unbound fraction. After centrifugation, the bound complex is then removed and suspended in scintillant fluid for counting. Each tissue sample is assayed at least twice in duplicate, and blanks and standards are included in each run.

The kits used for the assay were the cyclic AMP RIA kit (The Radiochemical Centre, Amersham, Buckinghamshire).

Cyclic GMP

These assays were also performed by Miss Cecile Muller using the cyclic GMP RIA kit, also supplied by the Radiochemical Centre. The method is based on the same principles underlying the cyclic AMP assay.

Activities of Ca^{++}/Mg^{++} actomyosin ATPase, Ca^{++} and K^{+}, EDTA myosin ATPase, Troponin-I and myosin P light chain phosphorylation.

In collaboration with Ms. Thérèse Resink and Professor W. Gevers of the Department of Medical Biochemistry, University of Cape Town, studies were performed to determine whether differences in these parameters could

explain differences in isolated heart function between trained and control rats.

These studies, which were performed as part of Ms. Resink's Ph.D. thesis, are also included in this thesis with the permission of those workers. The biochemical methods for these assays are described in detail in papers by Resink and Gevers[482-484,487] and are not re-produced here as they were not performed by this author.

Efficiency of biochemical methods.

Standards and blanks were run with each assay to determine the efficiency of each assay procedure.

E. Calibration procedures for the pressure and flow transducers.

The Statham P23 aortic and left ventricular pressure transducers used in sections 5.2 and 6.3 were calibrated in steps of 50 mmHg from 0 to 200 mmHg at the beginning and during the course of each experimental day. For the dP/dt calibrations, a calibrated voltage ramp was applied to the left ventricular pressure amplifier input. The output of this amplifier produced a deflection on the paper recorder (channel A) and was simultaneously fed into a differentiator channel which also produced a deflection on the paper recorder (Channel B). Hence a known rate of voltage change shown on Channel A produced a square wave on the differentiator trace (Channel B), equivalent to the rate of change of voltage on Channel A. Since the pressure trace was calibrated in mmHg, the amplitude of the ramp could be interpreted in mmHg. Hence the square wave on the differentiated trace could be read as a rate of pressure change in mmHg/sec.

In those experiments (section 6.4) in which aortic flows, and aortic and left ventricular pressures were analyzed by the minicomputer, the calibration procedures were as follows:

1) A least squares regression line was fitted to the analogue and directly-measured values for aortic flow when the aortic flow was zero, and for 5 forward and 2 reverse flows.

Aortic flows were measured directly by collecting 30 second samples of the fluid passing through the flow probe. During the calibration procedures, the aortic flow came from the Langendorff reservoir (Fig. 5.1), the fluid level of which was maintained at a constant height whilst the calibration was in progress. The flows, so measured, ranged between -60 and +220 ml/min.

2) The National Semiconductor aortic and left ventricular pressure transducers were calibrated simultaneously against a common pressure, in steps of 50 mmHg from 0-200 mmHg. A least squares regression equation line was also fitted to these data.

Both calibration procedures were performed at the beginning and end of each day's experiments. Any drift that occurred between calibrations, was apportioned to each experiment on the basis of when in the day, that experiment had been performed.

F. Calculation and expression of results and statistical methods.

Cardiac outputs (sum of the aortic outputs and coronary flow rates), stroke volumes (cardiac outputs divided by heart rates), and rates of coronary flow and myocardial oxygen consumption were all expressed relative to left ventricular (L.V.) dry weights. Biochemical data were related to left ventricular fresh weights. To facilitate the understanding of how the calculations listed below fit into the experimental protocols,

the reader is referred to the appropriate sections for which these calculations are appropriate.

Left ventricular dry weights (g) were calculated as the:

$$\text{Total LV wet weight} \times \frac{\text{dry weight of apex}}{\text{wet weight of apex}} \times \frac{100}{1}$$

Left ventricular fresh weights (g) were calculated as:

LV dry weight x 5 (Reference 477)

Myocardial oxygen consumption rates ($\mu l O_2/g/min$) were calculated as:

$$\frac{(P_aO_2 - P_vO_2) \times 0.031 \times \text{coronary flow (ml/min)}}{\text{LV dry weight}}$$

where P_aO_2 equals the oxygen tension of fluid entering the left atrium, and P_vO_2 equals the oxygen tension of the coronary effluent sampled under vacuum from the pulmonary artery. The technique whereby these samples were collected is described in the text (Appendix 1.C).

Note that myocardial oxygen consumption rates are expressed relative to dry weight. In order to correct to rates per gram fresh weight, the values should be divided by 5.

Substrate uptakes ($\mu M/g/min$) were calculated as:

$$\frac{S_5 - S_{65}\ (mM) \times \text{circulating volume (ml)}}{\text{LV fresh weight (g)} \times \text{perfusion time (min)}}$$

where S_5 equals the initial, and S_{65} the final perfusate substrate concentrations, as described in section 5.2B. The subscripts $_5$ and $_{65}$ indicate that the perfusate was samples at 5 and 65 minutes respectively, after working heart perfusion had commenced.

Glycogen utilization rates.

In studies of substrate metabolism, glycogen utilization rates (μM glucose equiv/g/min) were calculated as:

$$\frac{G_0 - G_{70}}{\text{perfusion time (min)}}$$

where G_0 equals the initial and G_{70} the final myocardial glycogen concentration. G_0 was taken as the mean glycogen value in a series of hearts clamped after 15 minutes' Langendorff perfusion (Table 5.5), and G_{70} was the myocardial glycogen content measured in each heart after 70 minutes' left atrial perfusion.

In studies during which $6{,}5 \times 10^{-7}$M isoproterenol was infused (sections 5.4 and 6.4) the mean glycogen utilization during isoproterenol infusion was calculated as:

$$\frac{G_{15} - G_{40}}{25}$$

where G_{15} equalled the mean myocardial glycogen content measured in hearts clamped after 15 minutes' left atrial perfusion, and G_{40} the mean myocardial glycogen content in a separate series of working hearts perfused for a further 25 minutes during which isoproterenol was infused.

Calculated rates of glycolytic flux (μmol. glucose equiv/g/min) were calculated from the rates of entry of C_6 units into glycolysis. This equalled the sum of the rates of glucose uptake and of glycogen utilization.

Calculated rates of glycolytic ATP production - non-radioactive method (μM ATP/g/min).

As each C_6 unit from glucose provides 2, and each C_6 unit from glycogen, 3 molecules of glycolytic ATP, glycolytic ATP production rates were calculated as the sum of twice the glucose uptake rates and three times the glycogen utilization rates.

Rates of lactate release (μmol/g/min) were calculated as:

$$\frac{(L_{65} - L_5) \times \text{circulating volume (ml)}}{\text{perfusion time (min)} \times \text{LV fresh weight}}$$

where L_{65} equals the final, and L_5 the starting perfusate lactate concentration.

Rate of C_6 oxidation (μmol./g/min) were calculated as:

$$\frac{(2 \times \text{calculated rate of glycolytic flux}) - (\text{rate of lactate release})}{2}$$

Absolute glycolytic rates - radioactive method (μmol. glucose eqiv/min) were calculated according to the following formula provided by Professor Rovetto (vide supra):

Absolute glycolytic rate (μmol glucose equiv/min) =

$$\frac{\left[\frac{\text{CPM (coronary effluent sample)}}{\text{sample size (0,5 ml)}}\right] - \text{CPM(blank)} \times \text{coronary flow rate} \quad \text{(ml/min)}}{\left[\frac{\text{CPM (Standard)} - \text{CPM (blank)}}{\text{Sample size (0,5 ml)}}\right] \times \text{perfusate glucose concentration (11,1 μmoles/ml)}}$$

Dividing this answer by the L.V. fresh weight gave glycolytic rates/g. Rates of glycolytic ATP production were then calculated from the glycolytic rates and rates of glycogen utilization, using the formula described previously.

Circulating volumes (ml) were calculated as:

$$\text{Circulating volume (ml)} = \frac{\text{CPM (standard) x 100}}{\text{CPM}(H_{65}-H_5) - \text{CPM (blank)}}$$

Calculation of data from the left ventricular pressure traces.

Peak left ventricular and aortic pressures (mmHg) were read from the appropriate points on the graphs.

End-diastolic pressures (mmHg) were taken as the highest left ventricular pressures recorded immediately preceding the rapid systolic upstroke on the left ventricular traces.

Aortic ejection times (msec) were taken as the time during which the aortic valve was open, as indicated from the upstroke to the dicrotic notch on the aortic pressure traces.

Left ventricular relaxation times (msec) were taken as the times from when LV dP/dt was 0 and decreasing through its maximum negative value (LV max -ve dP/dt) to the last point when LV dP/dt was -480 mmHg/sec and becoming positive[261].

Left ventricular maximum positive and maximum negative dP/dt values were taken as the maximum positive and negative deflections on the differentiated traces.

Heart work (mW/g LV dry wt) and efficiency (joules/ml O_2) were calculated in some experiments, according to the formulae derived by Kannengiesser, Opie and van der Werff[457].

Computer techniques.

In those experiments in which the computer was used to analyze left ventricular function (Chapter 6), data reduction was performed by programmes written by Dr. T.J. van der Werff (Department of Biomedical Engineering, University of Cape Town), who supplied the following details[458]:

A suite of computer programmes was written to reconstitute the pressure and flow waveforms from the raw data for immediate visualisation on a Tektronics 4025 graphics terminal and to process the data subsequently.

Cycles.

The first phase of the data reduction procedure was to identify an integral number of cardiac cycles. The program found the first systolic flow peak and searched forward for a further ten peaks. Subsequent data analyses were performed on, and averaged over, the intervening 10 complete cycles. From the peak systolic flow data point for each cycle, the data were searched backwards and forwards to identify the beginning and end of systole. The onset of flow systole was defined as the first data point for which the aortic flow was positive. Similarly, the onset of flow diastole was defined as the first data point for which the aortic flow was negative after systole. The onset of pressure systole was defined as the first data point on the systolic upstroke which exceeded its

predecessor by 1 mmHg, corresponding to a rate of change of dP/dt greater than 155 mmHg/sec. The onset of pressure diastole was defined as the first data point after the dicrotic notch, which in turn was defined as the local minimum pressure. Heart rate was calculated by subtracting the onset time of the first systole from that of the eleventh, dividing this into 10 (cycles), and converting into beats per minute.

The left ventricular end diastolic pressure was defined as the last data point before a data point on the systolic upstroke exceeded its predecessor by 1 mmHg.

Exact calculations imply ideal measurements of aortic pressure and left ventricular outflow. The pressure was adequately sampled and may be considered ideal. However, the left ventricular outflow is slightly less than ideal. It is composed of an adequately sampled aortic flow and an estimated instantaneous coronary flow, because technical limitations allowed only mean coronary flow to be measured. The instantaneous coronary flow was defined as follows: (a) the coronary flow data points during diastole were set equal to the negative of the corresponding aortic flow data points since during diastole there is no net flow output from the heart and the coronary flow can only come from the aortic cannula:

$$Q_{cn} = -Q_{an} \qquad (1)$$

the subscript "n" designating the n-th data point; and (b) the coronary flow data points during systole were set proportional to the aortic pressure:

$$Q_{cn} = k\, P_n \qquad (2)$$

The constant K is set so that the total computed coronary flow matched its measured value. The mean flow, i.e. cardiac output, and the mean pressure were calculated from the data using

$$\bar{Q} = \frac{1}{N} \sum_{n=1}^{N} (Q_{an} + Q_{cn}), \qquad (3)$$

$$\bar{P} = \frac{1}{N} \sum_{n=1}^{N} P_n, \qquad (4)$$

where N is the number of data points in the ten cardiac cycles being analysed. Typically N = 220 - 360.

Power.

The total power output is calculated by averaging the instantaneous values of both pressure power ($\dot{W}_p$) and kinetic power ($\dot{W}_k$):

$$\dot{W}_t = \dot{W}_p + \dot{W}_k = \frac{1}{N} \sum_{n=1}^{N} (P_n Q_n + \rho Q_n^3/2A^2). \qquad (5)$$

Appropriate conversion factors were included in all calculations so that power had the units of milliwatts, pressure mmHg, and flow ml/min.

dP/dt

Numerical differentiation of the left ventricular pressure was performed by applying the five-point approximation formula[514] at each data point:

$$dP_i/dt = (P_{i-2} - 8P_{i-1} + 8P_{i+1} - P_{i+2})/12\Delta t, \qquad (6)$$

where Δt = 6,48 msec is the time step between data points. This approximation given an error of order $(\Delta t)^4$, i.e. is very accurate for the well behaved left ventricular pressures.

Each cycle was searched to find the maximum and minimum (maximum negative) values of dp/dt. Because dp/dt changes rapidly we fit parabolas through the "apparent" maxima and minima to obtain the magnitudes and times of the "true" maxima and minima. Let t_m denote the time and f_m denote the magnitude of the apparent maximum (minimum). Define an auxiliary variable as follows:

$$F = (f_{m+1} - f_{m-1})/(f_{m+1} - 2f_m + f_{m-1}). \tag{7}$$

Then the "true" time t_m^* and magnitude f_m^* are

$$t_m^* = t_m - \tfrac{1}{2}F\Delta t, \tag{8}$$

$$f_m^* = f_m - \tfrac{1}{4}F(f_{m+1} - f_{m-1}). \tag{9}$$

Relaxation time.

The relaxation time was defined as the time interval between the left ventricular pressure derivative becoming zero and its minimum value. The "true" zero crossing time t_k^* was calculated by linear interpolation from the first negative data point (t_k) and its predecessor:

$$t_k^* = t_{k-1} + \Delta t\, f_{k-1}/(f_{k-1} - f_k). \tag{10}$$

The relaxation time was then simply the difference between the time of the minimum as calculated from Equation (8) for that cycle and the zero crossing time from Equation (10).

Statistical methods.

Results are expressed as mean values ± standard errors of the mean. The Student's unpaired t test was used to compare means between groups. A probability (P) value of less than 0,05 was considered to indicate a significant difference between mean values.

APPENDIX 2

Left ventricular functions of hearts perfused with the different substrate combinations under conditions of changing atrial filling pressures and heart rates.

Tables of results.

APPENDIX 2.1

STROKE VOLUMES (ml/g) OF HEARTS PERFUSED WITH DIFFERENT SUBSTRATE COMBINATIONS UNDER CONDITIONS OF CHANGING ATRIAL FILLING PRESSURES AND HEART RATES.

SUBSTRATE COMBINATION	HEART RATE - 330 BEATS/MIN ATRIAL FILLING PRESSURE (cm H_2O)				ATRIAL FILLING PRESSURE - 25 cm H_2O HEART RATE (BEATS/MIN)		
(No. of hearts)	15	20	25	30	300	360	390
1mM Palmitate (3% Albumin) (8)	1,38 ±0,10	1,55 ±0,09	1,50 ±0,14	1,13 ±0,12	1,49 ±0,15	1,20 ±0,13	1,07 ±0,09
11 mM Glucose (3% Albumin) (7)	1,34 ±0,07	1,59 ±0,11	1,66 ±0,09	1,54 ±0,16	1,66 ±0,11	1,57 ±0,12	1,46 ±0,12
11 mM Glucose (10)	1,55 ±0,03	1,84 ±0,05	1,90 ±0,08	1,61 ±0,12	1,94 ±0,10	1,72 ±0,09	1,48 ±0,09
10 mM Lactate (7)	1,38 ±0,06	1,60 ±0,07	1,80 ±0,07	1,78 ±0,10	1,99 ±0,10	1,61 ±0,07	1,48 ±0,09
11 mM Glucose 10 mM Lactate Insulin 2 U/L (7)	1,76 ±0,06	2,03 ±0,08	2,20 ±0,09	2,04 ±0,14	2,22 ±0,13	2,06 ±0,09	1,86 ±0,12
10 mM Pyruvate 11 mM Glucose Insulin 2 U/L (6)	1,70 ±0,09	1,92 ±0,09	1,93 ±0,07	1,70 ±0,12	1,94 ±0,06	1,74 ±0,11	1,56 ±0,13
11 mM Glucose Insulin 2 U/L (7)	1,58 ±0,09	1,99 ±0,04	2,16 ±0,04	2,12 ±0,04	2,32 ±0,05	2,06 ±0,05	1,91 ±0,06
11 mM Glucose 1 mM Palmitate Insulin 2 U/L (3% Albumin) (6)	1,60 ±0,09	1,89 ±0,05	2,00 ±0,08	1,80 ±0,17	2,09 ±0,06	1,83 ±0,08	1,62 ±0,06

APPENDIX 2.2

CORONARY FLOW RATES (ml/g/min) OF HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS UNDER CONDITIONS OF CHANGING ATRIAL FILLING PRESSURES AND HEART RATES.

SUBSTRATE COMBINATION	HEART RATE - 330 BEATS/MIN ATRIAL FILLING PRESSURE (cm H_2O)				ATRIAL FILLING PRESSURE - 25 cm H_2O HEART RATE (BEATS/MIN)		
	15	20	25	30	300	360	390
1 mM Palmitate (3% Albumin)	205,5 ±6,8	218,1 ±10,0	212,8 ±9,4	206,3 ±5,9	212,0 ±7,4	205,9 ±8,6	209,3 ±4,3
11 mM Glucose (3% Albumin)	181,7 ±12,4	200,3 ±8,7	203,6 ±5,0	198,4 ±4,5	201,5 ±3,7	209,0 ±3,0	207,5 ±4,2
11 mM Glucose	202,5 ±8,3	209,1 ±6,0	211,7 ±7,0	215,8 ±9,3	205,1 ±6,3	215,9 ±6,9	217,0 ±6,9
10 mM Lactate	194,2 ±10,0	212,2 ±10,7	214,8 ±5,5	227,0 ±6,4	225,3 ±9,4	221,8 ±4,7	219,8 ±5,3
11 mM Glucose 10 mM Lactate Insulin 2 U/L	216,1 ±9,0	226,2 ±8,6	226,6 ±9,2	225,3 ±8,6	220,3 ±9,8	225,6 ±10,4	225,2 ±11,6
10 mM Pyruvate 11 mM Glucose Insulin 2 U/L	185,4 ±13,6	205,2 ±12,2	206,7 ±10,5	198,1 ±15,1	199,5 ±9,2	199,2 ±11,0	196,7 ±14,8
11 mM Glucose Insulin 2 U/L	204,2 ±8,7	209,9 ±6,9	220,0 ±6,2	229,7 ±6,8	220,4 ±5,8	227,8 ±6,1	233,0 ±5,9
11 mM Glucose 1 mM Palmitate Insulin 2 U/L (3% Albumin)	212,1 ±8,7	248,9 ±7,0	253,3 ±8,3	247,4 ±14,5	252,9 ±8,5	253,4 ±12,0	252,4 ±12,0

APPENDIX 2.3

PEAK LEFT VENTRICULAR SYSTOLIC PRESSURES (mmHg) OF HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS UNDER CONDITIONS OF CHANGING ATRIAL FILLING PRESSURES AND HEART RATES, DURING NORMAL AND ISOVOLUMIC BEATS.

SUBSTRATE COMBINATION	HEART RATE - 330 BEATS/MIN ATRIAL FILLING PRESSURE (cm H_2O)								ATRIAL FILLING PRESSURE - 25 cm H_2O HEART RATE (BEATS/MIN)					
	15		20		25		30		300		360		390	
	N*	ISO**	N	ISO	N	ISO	N	ISO	N	ISO	N	ISO	N	ISO
1 mM Palmitate (3% Albumin)	159,3 ±4,7	184,3 ±5,0	160,1 ±4,8	181,1 ±4,0	155,9 ±3,5	178,2 ±5,6	140,6 ±5,4	157,2 ±7,0	158,9 ±4,9	180,7 ±4,8	142,3 ±4,3	158,9 ±5,1	133,6 ±3,1	154,4 ±5,4
11 mM Glucose (3% Albumin)	160,8 ±3,6	194,5 ±8,0	169,9 ±3,1	195,9 ±7,7	172,1 ±5,5	199,5 ±5,6	166,7 ±4,9	195,9 ±8,6	166,9 ±5,9	205,5 ±5,8	166,7 ±4,0	194,9 ±7,0	161,3 ±3,2	181,1 ±6,6
11 mM Glucose	168,1 ±5,2	188,7 ±4,3	168,6 ±5,7	187,7 ±5,5	169,5 ±6,3	188,3 ±4,9	160,0 ±5,8	187,8 ±5,1	173,8 ±5,0	184,5 ±7,7	165,0 ±5,9	178,2 ±4,6	157,8 ±7,0	169,5 ±5,9
10 mM Lactate	146,5 ±5,6	186,6 ±4,3	151,2 ±3,6	181,1 ±2,9	151,2 ±4,3	174,2 ±2,3	152,1 ±5,3	175,6 ±3,6	154,0 ±4,5	181,7 ±3,1	148,0 ±4,0	168,7 ±4,5	143,4 ±3,9	156,2 ±5,1
11 mM Glucose 10 mM Lactate Insulin 2 U/L	172,8 ±3,0	219,8 ±3,6	176,0 ±3,3	214,8 ±4,4	172,4 ±2,2	207,0 ±3,0	167,7 ±3,1	203.2 ±3,9	173,7 ±1,8	212,4 ±3,3	169,1 ±2,5	195,4 ±4,0	162,2 ±3,3	181,6 ±5,8
10 mM Pyruvate 11 mM Glucose Insulin 2 U/L	176,8 ±4,5	232,9 ±7,3	176,9 ±5,4	222,6 ±9,7	175,8 ±5,1	221,0 ±9,0	168,8 ±6,0	202,7 ±14,4	175,8 ±3,6	226,3 ±8,0	169,4 ±5,6	212.9 ±11,0	162,4 ±6,4	194,6 ±7,3
11 mM Glucose Insulin 2 U/L	163,7 ±4,0	200,5 ±5,8	176,6 ±3,5	204,8 ±4,0	177,2 ±4,0	205,4 ±4,5	178,7 ±3,9	205,1 ±5,2	180,6 ±3,8	210,8 ±3,6	176,6 ±3,3	204,3 ±4,1	174,1 ±3,2	197,9 ±4,8
11 mM Glucose 1 mM Palmitate Insulin 2 U/L (3% Albumin)	187,5 ±6,3	211,4 ±6,3	189,6 ±4,8	218,0 ±7,0	195,7 ±4,9	204,7 ±4,9	176,9 ±9,9	196,5 ±8,3	188,9 ±5,1	214,2 ±4,5	179,3 ±5,3	206,0 ±6,6	178,7 ±7,2	186,8 ±3,0

* Normal ** Isovolumic

APPENDIX 2.4

LEFT VENTRICULAR END-DIASTOLIC PRESSURES (mmHg) OF HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS UNDER CONDITIONS OF CHANGING ATRIAL FILLING PRESSURES AND HEART RATES.

SUBSTRATE COMBINATION	HEART RATE - 330 BEATS/MIN ATRIAL FILLING PRESSURE (cm H_2O)				ATRIAL FILLING PRESSURE - 25 cm H_2O HEART RATE (BEATS/MIN)		
	15	20	25	30	300	360	390
l mM Palmitate (3% Albumin)	12,0 ±0,9	16,9 ±0,9	16,5 ±0,7	16,3 ±1,1	17,6 ±1,3	16,3 ±0,4	15,6 ±0,5
l1 mM Glucose (3% Albumin)	13,2 ±1,3	14,3 ±1,9	15,6 ±2,0	14,5 ±1,6	17,6 ±1,5	16,0 ±1,5	14,9 ±1,6
l1 mM Glucose	9,7 ±0,4	13,2 ±0,9	15,2 ±0,9	12,4 ±1,1	15,6 ±0,6	13,9 ±0,8	12,7 ±1,0
.0 mM Lactate	10,6 ±0,6	11,5 ±1,0	15,4 ±1,4	14,7 ±1,4	14,7 ±1,0	13,1 ±1,1	12,9 ±1,2
.1 mM Glucose .0 mM Lactate nsulin 2 U/L	11,1 ±1,1	13,6 ±1,1	15,4 ±1,0	15,5 ±1,3	17,2 ±0,7	16,1 ±0,1	14,3 ±0,9
.0 mM Pyruvate .1 mM Glucose nsulin 2 U/L	12,9 ±1,0	17,5 ±0,7	19,7 ±0,7	17,1 ±0,3	19,6 ±1,1	16,7 ±0,9	16,7 ±1,2
.1 mM Glucose nsulin 2 U/L	9,1 ±0,3	11,5 ±0,6	14,7 ±1,0	16,3 ±0,9	15,7 ±0,4	13,8 ±0,8	12,4 ±0,9
.1 mM Glucose . mM Palmitate nsulin 2 U/L 3% Albumin)	11,7 ±0,6	16,4 ±1,1	17,8 ±0,8	20,7 ±1,0	19,0 ±0,8	16,5 ±0,9	15,4 ±0,8

APPENDIX 2.5

CALCULATED MYOCARDIAL OXYGEN CONSUMPTION RATES ($\mu l O_2/g/min$) OF HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS UNDER CONDITIONS OF CHANGING ATRIAL FILLING PRESSURES AND HEART RATES.

SUBSTRATE COMBINATION	HEART RATE - 330 BEATS/MIN ATRIAL FILLING PRESSURE (cm H_2O)				ATRIAL FILLING PRESSURE - 25 cm H_2O HEART RATE (BEATS/MIN)		
	15	20	25	30	300	360	390
1 mM Palmitate (3% Albumin)	2041 ±139	2344 ±138	2222 ±153	2117 ±127	2097 ±171	2158 ±114	2261 ±152
11 mM Glucose (3% Albumin)	1671 ±75	1868 ±110	2035 ±106	1941 ±102	1896 ±138	2041 ±151	2051 ±143
11 mM Glucose	1862 ±98	2043 ±79	2101 ±69	2092 ±113	2018 ±89	2183 ±112	2181 ±130
10 mM Lactate	2019 ±118	2307 ±134	2340 ±75	2453 ±113	2522 ±166	2592 ±123	2653 ±176
11 mM Glucose 10 mM Lactate Insulin 2 U/L	2211 ±72	2387 ±89	2424 ±76	2457 ±127	2322 ±109	2458 ±112	2475 ±132
10 mM Pyruvate 11 mM Glucose Insulin 2 U/L	1870 ±161	2164 ±172	2199 ±129	2130 ±147	2022 ±51	2137 ±129	2118 ±172
11 mM Glucose Insulin 2 U/L	1924 ±107	2215 ±76	2410 ±80	2380 ±95	2328 ±74	2480 ±71	2507 ±116
11 mM Glucose 1 mM Palmitate Insulin 2 U/L (3% Albumin)	2076 ±134	2399 ±78	2498 ±122	2485 ±121	2411 ±121	2560 ±99	2606 ±83

APPENDIX 2.6

CALCULATED HEART WORK (mW) OF HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS UNDER CONDITIONS OF CHANGING ATRIAL FILLING PRESSURES AND HEART RATES.

SUBSTRATE COMBINATION	HEART RATE - 330 BEATS/MIN ATRIAL FILLING PRESSURE (cm H_2O)				ATRIAL FILLING PRESSURE - 25 cm H_2O HEART RATE (BEATS/MIN)		
	15	20	25	30	300	360	390
mM Palmitate 3% Albumin)	127,9 ±11,6	146,1 ±11,6	141,6 ±15,6	95,8 ±11,6	127,0 ±16,2	115,4 ±15,3	106,3 ±11,1
1 mM Glucose 3% Albumin)	114,1 ±7,6	129,7 ±8,4	154,2 ±14,0	142,2 ±20,0	139,5 ±13,7	156,0 ±16,9	154,2 ±15,0
1 mM Glucose	143,1 ±6,3	176,9 ±6,0	180,1 ±9,6	148,7 ±12,7	172,3 ±9,4	175,1 ±11,1	156,1 ±12,3
0 mM Lactate	112,4 ±5,3	139,4 ±7,8	161,3 ±8,8	161,5 ±13,8	166,9 ±14,3	155,9 ±11,2	149,3 ±14,1
1 mM Glucose 0 mM Lactate nsulin 2 U/L	180,2 ±7,7	215,0 ±11,3	232,2 ±12,1	211,3 ±19,8	218,4 ±16,9	235,9 ±16,5	233,0 ±21,1
0 mM Pyruvate 1 mM Glucose nsulin 2 U/L	173,5 ±12,4	204,3 ±15,0	206,5 ±16,6	170,5 ±17,1	188,0 ±9,8	194,9 ±16,1	181,2 ±19,9
1 mM Glucose nsulin 2 U/L	150,3 ±7,7	202,5 ±4,6	225,6 ±7,1	220,9 ±6,6	223,5 ±7,8	235,2 ±8,0	231,0 ±10,0
1 mM Glucose mM Palmitate nsulin 2 U/L 3% Albumin)	147,6 ±7,5	178,0 ±6,0	191,1 ±7,1	165,0 ±18,0	181,9 ±4,5	183,4 ±6,7	171,6 ±5,7

APPENDIX 2.7

CALCULATED MYOCARDIAL EFFICIENCIES (Joules/ml O_2) OF HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS UNDER CONDITIONS OF CHANGING ATRIAL FILLING PRESSURES AND HEART RATES.

These data can be found in Table 5.8.

APPENDIX 2.8

MAXIMUM RATES OF LEFT VENTRICULAR PRESSURE DEVELOPMENT (L.V. max. +ve dP/dt - mmHg/sec) OF HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS UNDER CONDITIONS OF CHANGING ATRIAL FILLING PRESSURES AND HEART RATES, DURING NORMAL AND ISOVOLUMIC BEATS.

SUBSTRATE COMBINATION	HEART RATE - 330 BEATS/MIN ATRIAL FILLING PRESSURE (cm H_2O)								ATRIAL FILLING PRESSURE - 25 cm H_2O HEART RATE (BEATS/MIN)					
	15		20		25		30		300		360		390	
	N*	ISO**	N	ISO	N	ISO	N	ISO	N	ISO	N	ISO	N	ISO
1 mM Palmitate (3% Albumin)	3560 ±37	3620 ±39	3530 ±44	3530 ±51	3500 ±45	3520 ±45	3280 ±102	3234 ±129	3480 ±59	3500 ±58	3350 ±58	3270 ±95	3246 ±39	3200 ±70
11 mM Glucose (3% Albumin)	3520 ±55	3680 ±51	3560 ±77	3680 ±62	3573 ±87	3653 ±79	3533 ±78	3627 ±67	3547 ±76	3699 ±56	3547 ±61	3640 ±54	3547 ±45	3547 ±61
11 mM Glucose	3568 ±21	3640 ±18	3568 ±21	3584 ±26	3504 ±31	3600 ±24	3512 ±22	3576 ±21	3528 ±25	3576 ±41	3488 ±30	3534 ±40	3464 ±31	3488 ±38
10 mM Lactate	3440 ±72	3589 ±51	3554 ±49	3623 ±38	3474 ±60	3543 ±52	3486 ±60	3543 ±38	3520 ±58	3577 ±60	3474 ±58	3463 ±57	3360 ±72	3383 ±76
11 mM Glucose 10 mM Lactate Insulin 2 U/L	3749 ±27	3851 ±32	3749 ±27	3840 ±30	3737 ±29	3806 ±24	3726 ±34	3771 ±27	3726 ±30	3829 ±27	3703 ±15	3749 ±32	3669 ±27	3680 ±35
10 mM Pyruvate 11 mM Glucose Insulin 2 U/L	3920 ±25	4064 ±47	3920 ±29	3973 ±61	3906 ±48	3947 ±61	3813 ±82	3853 ±106	3893 ±17	4013 ±38	3867 ±49	3907 ±78	3800 ±68	3787 ±76
11 mM Glucose Insulin 2 U/L	3554 ±46	3669 ±41	3646 ±16	3703 ±23	3623 ±23	3669 ±32	3634 ±24	3680 ±21	3634 ±24	3703 ±23	3634 ±24	3680 ±18	3600 ±25	3646 ±34
11 mM Glucose 1 mM Palmitate Insulin 2 U/L (3% Albumin)	3653 ±17	3747 ±25	3653 ±17	3720 ±18	3653 ±27	3680 ±21	3560 ±74	3613 ±86	3653 ±17	3733 ±17	3613 ±32	3640 ±27	3560 ±18	3587 ±32

* Normal ** Isovolumic

APPENDIX 2.9

MAXIMUM RATES OF LEFT VENTRICULAR RELAXATION (L.V. max. -ve dP/dt - mmHg/sec) OF HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS UNDER CONDITIONS OF CHANGING ATRIAL FILLING PRESSURES AND HEART RATES, DURING NORMAL AND ISOVOLUMIC BEATS.

SUBSTRATE COMBINATION	HEART RATE - 330 BEATS/MIN ATRIAL FILLING PRESSURE (cm H_2O)								ATRIAL FILLING PRESSURE - 25 cm H_2O HEART RATE (BEATS/MIN)					
	15		20		25		30		300		360		390	
	N *	ISO**	N	ISO	N	ISO	N	ISO	N	ISO	N	ISO	N	ISO
1 mM Palmitate (3% Albumin)	2880 ±67	3130 ±72	2780 ±64	3090 ±101	2610 ±108	2840 ±117	2366 ±128	2503 ±143	2430 ±108	2880 ±86	2590 ±98	2750 ±132	2605 ±58	2663 ±90
11 mM Glucose (3% Albumin)	2867 ±91	3067 ±156	2880 ±83	3053 ±56	2800 ±97	3080 ±96	2613 ±116	2840 ±68	2733 ±108	3000 ±96	2773 ±92	3027 ±98	2693 ±61	2840 ±113
11 mM Glucose	3064 ±92	3200 ±88	3080 ±62	3136 ±101	2896 ±72	2944 ±112	2656 ±66	2896 ±114	2864 ±65	2896 ±148	2912 ±91	2976 ±91	2792 ±108	2997 ±82
10 mM Lactate	2583 ±143	2971 ±86	2594 ±149	3061 ±94	2571 ±111	2971 ±111	2583 ±106	2869 ±105	2571 ±75	2923 ±94	2480 ±145	2823 ±134	2491 ±129	2789 ±133
11 mM Glucose 10 mM Lactate Insulin 2 U/L	2926 ±65	3497 ±54	2891 ±37	3406 ±63	2983 ±67	3234 ±107	2891 ±62	3097 ±97	2891 ±64	3211 ±62	3029 ±93	3211 ±62	2971 ±91	3189 ±59
10 mM Pyruvate 11 mM Glucose Insulin 2 U/L	2800 ±72	3576 ±179	2867 ±43	3360 ±197	2747 ±35	3267 ±177	2587 ±40	3187 ±215	2653 ±56	3293 ±208	2760 ±27	3253 ±193	2720 ±29	3093 ±151
11 mM Glucose Insulin 2 U/L	2857 ±119	3257 ±38	3109 ±86	3280 ±30	3074 ±80	3166 ±52	2926 ±24	3200 ±83	3074 ±107	3200 ±84	3051 ±51	3131 ±37	2994 ±65	3166 ±58
11 mM Glucose 1 mM Palmitate Insulin 2 U/L (3% Albumin)	2707 ±222	3253 ±76	2720 ±184	3212 ±84	2720 ±174	3120 ±85	2360 ±141	2787 ±73	2507 ±198	3027 ±63	2653 ±145	2987 ±84	2680 ±116	2960 ±88

* Normal ** Isovolumic

APPENDIX 2.10

RELAXATION TIMES (msec) OF HEARTS PERFUSED WITH THE DIFFERENT SUBSTRATE COMBINATIONS UNDER CONDITIONS OF CHANGING ATRIAL FILLING PRESSURES AND HEART RATES.

SUBSTRATE OMBINATION	HEART RATE - 330 BEATS/MIN ATRIAL FILLING PRESSURE (cm H_2O)				ATRIAL FILLING PRESSURE - 25 cm H_2O HEART RATE (BEATS/MIN)		
	15	20	25	30	300	360	390
mM Palmitate % Albumin)	76,4 ±1,1	81,4 ±2,3	84,8 ±2,0	88,6 ±4,0	86,3 ±1,6	86,9 ±1,7	84,3 ±1,2
1 mM Glucose 3% Albumin)	80,7 ±1,1	79,2 ±2,2	80,8 ±1,9	83,0 ±2,0	85,7 ±2,2	81,0 ±2,0	82,2 ±1,9
l mM Glucose	72,1 ±1,8	76,3 ±2,1	80,2 ±1,8	80,9 ±1,8	80,9 ±2,2	79,3 ±2,3	77,1 ±1,9
) mM Lactate	82,7 ±2,6	81,7 ±2,4	87,0 ±2,3	90,6 ±3,2	88,9 ±2,5	86,9 ±1,9	85,4 ±2,9
l mM Glucose) mM Lactate ısulin 2 U/L	72,1 ±0,9	75,3 ±0,8	80,4 ±0,9	82,4 ±1,0	81,4 ±1,5	79,6 ±0,8	78,0 ±1,1
) mM Pyruvate . mM Glucose ısulin 2 U/L	74,6 ±3,4	83,5 ±2,6	91,7 ±2,2	86,0 ±1,9	90,8 ±2,3	83,7 ±2,0	80,8 ±2,6
. mM Glucose ısulin 2 U/L	75,0 ±1,5	76,9 ±0,9	81,4 ±1,1	83,1 ±1,0	84,1 ±0,6	78,7 ±1,1	76,3 ±0,9
mM Glucose mM Palmitate sulin 2 U/L % Albumin)	73,2 ±1,2	76,7 ±1,3	80,0 ±1,4	81,5 ±1,3	82,5 ±1,0	78,8 ±1,6	76,3 ±0,8

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