# Gradients in myocardial blood flow, metabolism and mechanics across the ischemic left ventricular wall

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CONTENTS

## List of symbols and abbreviations

| | |
|---|---|
| ATP | adenosine triphosphate |
| AV (difference) | arterio-local venous difference ($mmol.l^{-1}$) |
| $\beta_{min}$ | angle of epicardial minimal shortening with the circumference (rad) |
| $\beta_{min,endo}$ | angle of minimal shortening in the inner layers with the circumference (rad) |
| $\gamma$ | shear angle (rad) |
| $dp_{lv}/dt\ max$ | maximal first positive derivative of left ventricular pressure ($kPa.s^{-1}$) |
| ECG | electrocardiogram |
| endo/epi ratio | endo/epicardial blood flow ratio |
| $e_c$ | circumferential shortening (%) |
| $e_{endo}$ | shortening in the inner layers in the direction of (epicardial) $e_{min}$ (%) |
| $e_{max}$ | maximal shortening on the epicardium (%) |
| $e_{max,endo}$ | maximal shortening in the inner layers (%) |
| $e_{min}$ | minimal shortening on the epicardium (%) |
| $e_{min,endo}$ | minimal shortening in the inner layers (%) |
| $e_z$ | base-to-apex shortening (%) |
| endo | inner layers (1/3) of the left ventricular wall |
| epi | outer layers (1/3) of the left ventricular wall |
| i.m. | intramuscular |
| i.v. | intravenous |
| LAICA | left anterior interventricular coronary artery |
| LCCA | left circumflex coronary artery |
| MFGC | magnetic field generating coil |
| MBF | myocardial blood flow |
| meso | middle layers (1/3) of the left ventricular wall |
| n | number of observations |
| NEFA | non-esterified fatty acids |
| $p_{ao}$ | aortic pressure |
| $p_{cor}$ | coronary artery pressure distal to the site of stenosis |
| $p_{lv}$ | left ventricular pressure |
| $p_{lved}$ | end-diastolic left ventricular pressure |

| pH | negative logarithm of hydrogen ion concentration |
|---|---|
| $p_{CO2}$ | partial carbondioxide tension |
| $p_{O2}$ | partial oxygen tension |
| s | second(s) |
| SC | sensor coil |

Tissue contents of the various substances are expressed as $mol.g^{-1}$ wet weight, unless explicitly mentioned otherwise.

1. GENERAL INTRODUCTION

It is well known that myocardial ischemia as a result of narrowing (steno-
sis) of a coronary artery is most pronounced in the subendocardial (or inner)
layers of the heart muscle. The greater vulnerability of the inner layers is
demonstrated not only by the significantly lower blood flow in these layers
during ischemia as compared with the other layers of the myocardium (Jageneau
et al, 1975; Reneman et al, 1975; Bache et al, 1977; Nakamura et al, 1977;
Schamhardt et al, 1979), but also by the biochemical changes in the different
layers under these circumstances. A significantly higher lactate content has
been found in the inner than in the outer layers after coronary artery ste-
nosis or occlusion. Also a more pronounced decrease of glycogen and creatine
phosphate in the inner than in the outer layers has been shown (Griggs et al,
1972; Opie et al, 1975; Opie and Owen, 1976; Higuchi et al, 1981). However,
assessment of tissue ATP contents showed contradictory results. Griggs et al
(1972) found that after coronary artery stenosis the decrease and the trans-
mural differences in ATP content were not statistically significant. In
contrast, Opie and Owen (1976) found a distinct decrease (90% or more) in ATP
concentration in the inner layers and a moderate decrease (only 30%) in the
outer layers. This discrepancy may result from differences in experimental
set-up and/or sample interval after the onset of ischemia. A time course of
tissue contents of ATP, creatine phosphate as well as of inorganic phosphate
in the venous effluence has been found (Opie et al, 1972), while Higuchi et
al (1981) showed that the decrease and the transmural gradient of ATP content
were dependent on the degree of ischemia.
    Beside changes in blood flow and metabolism, changes in regional mechani-
cal activity do occur following the inducement of myocardial ischemia (Ten-
nant and Wiggers, 1935; Hood et al, 1969; Van der Meer et al, 1973; Banka and
Helfant, 1974; Theroux et al, 1976). These studies, however, do not provide
data on differences in mechanical activity between inner and outer layers
under these circumstances. Consequently, it is not known whether there is a
relation between the decrease in blood flow and mechanical activity and the
metabolic changes in the various layers of the myocardium. Diminution or ces-
sation of mechanical activity could be directly related to factors inhibiting
this activity like high cellular concentrations of hydrogen ions (Katz and
Hecht, 1969; Poole Wilson and Langer, 1976), inorganic phosphate (Kuebler and
Katz, 1977) or fatty acids (Katz and Messineo, 1981), or depletion of energy-

rich phosphates (Gudbjarnason et al, 1970; Hearse, 1979) or glycogen (Rovet-to, 1979).

Moreover, it is important to know the time course of mechanical activity during the whole period of ischemia. A time-dependent decrease in local mechanical activity during this period could be responsible for the decrease in arterio-venous differences for potassium, lactate and inorganic phosphate as observed during stenosis of a coronary artery (Owen et al, 1970; Opie et al, 1972; Van der Vusse et al, 1980).

Energy for processes is achieved from the residual aerobic metabolism and anaerobic glycolysis. Because of the low oxygen uptake and the inefficient ATP production from anaerobic glycolysis under these circumstances, insufficient energy may be delivered to maintain cell viability during ischemia. Beside low ATP concentrations, depletion of glycogen stores and accumulation of lactate have been mentioned to coincide irreversable damage (Reimer et al, 1973, 1981; Jennings et al, 1981).

Abnormalities in lipid metabolism may be involved in cell death. Although during ischemia fatty acid combustion is impaired (Shug et al, 1978), fatty acids are still taken up from the blood (Opie et al, 1973). This could lead to increased tissue contents of triacylglycerol or non-esterified fatty acids (NEFA) (Lochner et al, 1978). NEFA accumulation could also origin from breakdown of phospholipids which are present in cellular membranes (Vasdev et al, 1980). The few data on this subject presented in literature are contradictory. No significant (Regan et al, 1972; Haider et al, 1977) and elevated NEFA contents (Weishaar et al, 1977, 1979; Van der Vusse et al, 1982) have been described following oxygen deficiency. Moreover, no clear information is available about the time course of this elevation during ischemia, and NEFA contents reported in these studies vary greatly in the normoxic as well as in the ischemic situation.

High NEFA contents are thought to be harmful for the ischemic myocardium. In vitro experiments with isolated enzymes and subcellular fractions have shown that high NEFA concentrations can inhibit enzyme activities and mito-chondrial energy production (Katz and Messineo, 1981). Elevated plasma NEFA concentrations are related to ventricular arrhythmias (Kurien and Oliver, 1966). It is not known whether this noxious effect also originates from increased intracellular NEFA contents under these circumstances or from a direct effect of the high plasma NEFA concentrations on membranes.

Knowledge about the relation between blood flow, metabolism and mechani-

cal activity in the various myocardial layers during ischemia is important.
- Firstly, to get insight into the mechanism(s) involved in the cessation of mechanical activity during ischemia.
- Secondly, this relation has never been studied extensively.
- Thirdly, it is known that without impairment of the coronary circulation, generated energy as well as blood flow per unit of tissue volume in the different layers of the myocardial wall depends on the hemodynamic load (Buckberg et al, 1972; Yipintsoi et al, 1973; Arts, 1978) and treatment of ischemic heart failure is generally employed by changing preload or after-load
- Fourthly, the metabolism of fatty acids during ischemia is important because accumulation of NEFA can either be a cause or a result of irreversible damage, as well as a cause of detrimental events for the whole heart.

The relation between blood flow, metabolism and mechanical activity in the various layers of the myocardium was investigated in an open-chest dog preparation. Regional ischemia was induced by narrowing the left anterior interventricular coronary artery was by an inflatable cuff. The degree of inflation of the cuff was controled by the post-stenotic coronary artery pressure, so that a reproducible degree of ischemia could be obtained. Blood flow within various layers of the normoxic and ischemic left ventricular myocardium was measured with the radioactive microsphere technique (reviewed by Heymann et al, 1977). Regional mechanical activity in the ischemic area was determined by measurement of epicardial surface deformation as described by Arts and Reneman (1980). Evidence will be shown that this technique provides relevant information about the shortening along the subendocardial fiber direction in the inner and outer layers. Metabolism in the normoxic and ischemic tissue was assessed by measurement of concentrations of ATP, creatine phosphate, glycogen and fatty acids in tissue samples as well as by measurement of differences in the concentrations of oxygen, glucose, fatty acids, lactate, inorganic phosphate and potassium between arterial blood and blood sampled from a local vein, that drains the ischemic area.

## 2. SURVEY OF THE LITERATURE

### 2.1 Mechanics

### 2.1.1 Global mechanics of the left ventricle and its transmural mechanical distribution.

The basic structure of the vertebrate heart muscle is characterized by muscle cells (fibers) arranged in columns (Spiro and Sonnenblick, 1965). Light microscopic examination reveals these cells to be roughly cylindrical in shape, measuring 10-20 $\mu$m in diameter and 50-100 $\mu$m in length (Hort, 1960). The myocardial wall is composed of a well ordened fiber-wound continuum of interconnected muscle fibers. Studies in the dog (Streeter et al, 1969) and macaque (Ross and Streeter, 1975) have shown that the orientation of the fibers is nearly vertical (i.e. perpendicular to the circumferential direction) at both epicardial and endocardial surfaces. In moving through the ventricular wall, fiber direction undergoes a marked change such that the angle made with the circumferential direction is about $60^0$ for the fibers near the inner surface, about $0^0$ for the fibers in the middle of the wall and about $-60^0$ for the fibers near the epicardial surface. Studies of Torrent Guasp (1973) showed that the above-mentioned orientation, as established by sampling tissue from a certain area of the left ventricle, is a result of the course of strings of fibers through the wall: roughly a three-dimensional eight-shaped curve.

This architecture of the left ventricle not only results in a predominant circumferential decrease of size during ejection, but also in rotation of the apex around the long axis of the ventricle with respect to the base during contraction. This movement, already observed by Leonardo da Vinci (Evans, 1936), is called torsion. Studies of Streeter et al (1970) showed the importance of the complex fiber orientation with respect to transmural fiber stress distribution. Moreover, Arts (1978) predicted from a mathematical model that fiber stress and fiber shortening (and therefore energy demand) are rather homogeneously distributed across the left ventricular wall. The latter homogeneity is enabled by the occurrence of torsion.

Attempts have been made to assess transmural distribution of workload from intramyocardial pressure measurements. Armour and Randall (1971) found a higher systolic pressure in the inner than in the outer layers but calculation of developed tension revealed that this tension was slightly lower in

the inner layers due to the smaller diameter of the ventricle in the inner than for the outer layers. However, Marzilli et al (1980, 1981) concluded from their data, that the work load in the inner layers would be considerably higher than in the outer layers due to a higher systolic intramyocardial pressure (200%), rate of rise of this pressure (270%), and shortening velocity (190%) as well as a more pronounced shortening in the inner layers. These results, however, should be interpretated with care, because measurement of intramyocardial pressure is subject to errors and generally the values are overestimated (see below), measured values of shortening highly depend on correct orientation of the ultrasonic crystals, e.g. parallel to the fiber direction, and last but not least, local workload is not related to intramyocardial pressure.

The above-mentioned findings seem to be in conflict with the findings that oxygen consumption in the inner layers is only 1.3-1.6 times higher than in the outer layers (Holtz et al, 1977; Weiss et al, 1978). These values, which may even be overestimations of the real transmural gradient (see below), suggest only a moderate transmural difference in workload.

## 2.1.2 Failure of mechanical activity during oxygen deprivation

After the onset of acute ischemia or anoxia, myocardial mechanical function is impaired within seconds (Tennant and Wiggers, 1935; Theroux et al, 1976; Lab et al, 1978; Hearse, 1979). Several hypotheses have been developed to explain this "early contractile failure".

A. Oxygen deprivation induced changes in cardiac contractile proteins (Katz and Hecht, 1969). This hypothesis is not very likely because even after prolonged ischemia no irreversible damage could be detected in the contractile proteins (Kako and Bing, 1958; Barany et al, 1964; Katz and Maxwell, 1964) or in the myofibrillar ATPase (Albert and Gordon, 1962).

B. Proton-induced changes in calcium homeostasis. The increased hydrogen ion concentration would interfere with the calcium binding to troponin (Katz and Hecht, 1969) or with mechanisms involved in the regulation of cytoplasmatic calcium concentrations (reviewed by Nayler et al, 1979). Several arguments have been raised against decreased pH as first responsible factor for the decline of contraction, such as:

a. the source of early proton production, if any, is not clear: neither carbondioxide nor anaerobic glycolysis are believed to cause a dramatic fall in pH in early ischemia (Opie, 1976; Gevers, 1977; Hearse, 1979).

Moreover, proton consuming creatine phosphate breakdown in the first moments after oxygen deprivation would rather cause a transient alkalosis (Danforth, 1965; Gevers, 1977).

b. although tissue pH measurements may still be debatable, these determinations did not reveal an impressive decline in pH when mechanical function decreased during ischemia (Poole-Wilson, 1975; Cobbe and Poole-Wilson, 1980).

c. comparing ischemia and hypoxia, Cobbe and Poole-Wilson (1980) showed that under the former circumstances, acidosis developped earlier and more severely, while the decline in tension development occurred at the same time in both cases.

C. Fatty acid induced changes in calcium homeostasis. Elevated concentrations of non-esterified fatty acids (NEFA), acyl-CoA or acylcarnitine could interfere with calcium influx during electrical activation as may be concluded from in vitro experiments (reviewed by Katz and Messineo, 1981). Higher contents of the afore-mentioned fatty acid-esters, as in non-working hearts from diabetic rats, coincided with a faster rate of ventricular failure of these hearts as compared with hearts from normal rats (Feuvray et al, 1979). Extrapolation of in vitro results to the myocardial cell in situ should be done with caution, because addition of albumin, a NEFA-binding protein, can abolish or greatly depress in vitro effects of NEFA (Borst et al, 1962). Specific binding proteins are known to be present in the myocardial cell (Mishkin et al, 1972; Ockner et al, 1972; Gloster and Harris, 1977). If high cellular NEFA concentrations would be harmful for the ischemic myocardium, the proposed deleterious effects should be preceeded by a NEFA accumulation. In chapter 6 the time course of the decrease in mechanical performance will be studied and the NEFA accumulation in chapter 7.

D. ATP depletion induced uncoupling of the excitation-contraction mechanism. After all, sudden oxygen deprivation will cause a decrease in ATP concentration. However, this simple and attractive hypothesis has been dismissed on the basis of reports which showed sufficient quantities of ATP when the contractile activity was severely reduced (Braasch et al, 1968; Katz, 1968; Williamson et al, 1976). Nevertheless, recently, arguments were found, which stress the importance of the degradation of energy-rich phosphates:

a.  relatively moderate decreases in ATP concentrations may already cause a
    reduction in contractility through the modulatory role of ATP in calcium
    movements (Kuebler and Katz, 1977).
b.  a relatively small decline in energy-rich phosphates yields considerable
    amounts of inorganic phosphate when compared with the normally occurring
    intracellular inorganic phosphate concentration. This could promote
    calcium precipitation, preventing calcium to react with the contractile
    proteins. Kuebler and Katz (1977) calculated an 8-fold increase in
    inorganic phosphate concentrations, while tissue contents have been
    shown to increase only 1.5 to 2-fold in the early phase of ischemia
    (Imai et al, 1979; Higuchi et al, 1981). The difference between calcula-
    ted and measured values might be due to the release of inorganic phos-
    phate from the ischemic myocardium (Opie et al, 1972; Van der Vusse et
    al, 1979). Moreover, the observation that during hypoxia the inorganic
    phosphate content hardly increased while developed tension declined
    (Dhalla et al, 1972) further invalidates this "phosphate trap" theory.
c.  ATP could exist in discrete cellular compartments and depletion of one
    small compartment, not detected by determination of the content in the
    whole biopsy, might be responsible for the decline in contractile energy
    (Gudbjarnason et al, 1970). These authors postulated inhibition of in-
    tracellular energy transfer in ischemic muscle by a breakdown of the
    unidirectional phosphocreatine energy shuttle. Also ATP compartments
    have been suggested to exist in the proximity of the contractile pro-
    teins (Yagi and Mase, 1965) and the cell membrane (Bricknell and Opie,
    1978). Existence of ATP compartments has become more likely because of
    the observation that ATP is produced by glycolysis and not the total ATP
    content is inversely related to development of contracture during ische-
    mia (Bricknell et al, 1981).
d.  Recently, in a preliminary report Kammermeier and coinvestigators (1981)
    suggested that a reduction in the free energy from ATP hydrolysis could
    be responsible for the early mechanical failure. This free energy is
    dependent on the logarithm of the phosphorylation potential:

$$\frac{[ATP]}{[ADP] \ [P_i]}$$

With impaired ATP synthesis and continuing energy consumption, even a
small decrease in [ATP] will lead to increases in ADP ([ADP]) and even
more in [Pi] by the rapid breakdown of creatine phosphate, and thus in

a marked decrease in the phosphorylation potential. This decrease might reach levels below the free energy per mole ($\Delta G$) needed by actomyosine ATPase for the reactions resulting in contraction. Indeed, Kammermeier et al (1981) showed that in the hypoxic non-working isolated perfused rat heart cessation of contraction paralleled the decline in the calculated free energy from ATP hydrolysis. In these calculations, they estimated the cytoplasmatic concentrations of the substances of interest as estimated by Giesen and Kammermeier (1980). Also in the isolated guinea pig heart, the phosphorylation potential (as calculated from the tissue contents) dramatically falls within half a minute after onset of ischemia, synchronously with the decrease in mechanical performance (Imai et al, 1979). This theory will be difficult to prove, because the cytosolic phosphorylation potential will control contractile activity, but only total tissue contents can be measured with the techniques presently available.

## 2.1.3 Measurement of regional mechanical variables

Parameters related to regional cardiac mechanics are (a) local fiber stress (or generated mechanical power per unit of tissue volume), (b) velocity of sarcomere shortening, (c) intramyocardial pressure, and (d) epicardial deformation. Assessment of regional left ventricular mechanics is difficult because of limitations of the measuring techniques for these parameters:

- in spite of several attempts (Feigl et al, 1967; Burns et al, 1971; McHale and Greenfield, 1973), direct determination of local wall stress remains difficult and unreliable because of artefacts like tissue injury at the site of measurement (Huisman et al, 1980).
- sarcomere length (Yoran et al, 1973; Grimm et al, 1980) and fiber orientation (Streeter and Hanna, 1973) can only be measured in the post-mortem heart.
- measurement of intramyocardial pressure is difficult and probably unreliable because the techniques used until now induce damage or disalignment of the contractile fibers (Arts and Reneman, 1977). Damage will enlarge the fluid cavity in which the intramyocardial pressure is measured resulting in an underpressure within the cavity causing an underestimation of intramyocardial pressure. Disalignment will increase the pressure in this compartment as a consequence of La Place's law.

In the absence of a direct and accurate technique to measure transmural

distribution of fiber stress and intramyocardial pressure, in the present study regional myocardial fiber mechanics are assessed from left ventricular wall deformation, as assessed on the epicardial surface.

## 2.1.4 Measurement of epicardial deformation

Determination of epicardial deformation during systole is useful in study- ing the process of cardiac contraction (Fenton et al, 1978; Lab and Woollard, 1978; Arts, 1978; Arts et al, 1980 and 1982b). During ejection of the left ventricle, locally measured circumferential and axial strain determine the decrease of the epicardial surface which is directly related to the contribu- tion of local myocardial tissue to total pump function of the left ventricle (Arts et al, 1982b). Epicardial shear is related to torsion of the left ventricle which is defined as a rotation of the apex around the axis of the left ventricle with respect to the base. Analysis of the mechanics of the left ventricle during ejection in a mathematical model revealed that the transmural distribution of fiber shortening was related to the amount of torsion of the left ventricle. In vivo measurements of epicardial shear (Arts et al, 1982b) compared favorably with the calculated values of these parame- ters. Therefore, it is likely, that the amount of torsion of the left ventri- cle determines the transmural distribution of fiber shortening. Thus, indi- rectly, information can be obtained about the transmural contractile behavior by measuring strains and shear angle at the surface of the heart.

Techniques to measure segment length on the heart are: (a) the determi- nation of the distance between opaque markers, implanted on the epicardial surface using X-ray film or video recording techniques (Carlsson, 1969; Fenton et al, 1978), and (b) measurement of epicardial segment length by strain gauges (Lab and Woollard, 1978), inductive coils (Irisawa et al, 1959; Wilson et al, 1963; Guntheroth, 1969; Hinds et al, 1969), or a pair of piezo- electric crystals, measuring the transit time of ultrasound which is propor- tional to the distance between the crystals (Rushmer et al, 1956; Stegal et al, 1967).

Recently, Arts and Reneman (1980) described a new method for the assess- ment of epicardial deformation, which is basically an inductive technique. Advantages of this technique are low noise, high sensitivity and good stabi- lity. Natural strains were derived electronically from changes in the ampli- tude of the signals induced in sensor coils. Moreover, application in open- chest experiments is easy because the inductive coils are simply sucked on

the epicardium.

## 2.2 Energetics

### 2.2.1 Myocardial heat production

This subject has been extensively reviewed by Gibbs (1978). The mechanical energy performed by the left ventricle is a result of metabolic processes in which the first law of thermodynamics is applicable. The total energy produced by the heart can thus be assessed by investigating the sum of external power and heat production. Global ventricular heat production can be reliably estimated through calculation of the product of coronary blood flow and the arterio-venous temperature difference while correcting for the fraction of heat that is not carried away by the coronary blood but dissipated to ventricular cavities, mediastinum and lungs as well as the small fraction that is lost through endothermic reactions in the coronary blood (Bui-MongHung et al, 1981; Ten Velden et al, 1982).

For the purpose of the present study, measurement of local heat production within 3 layers of the ventricular wall is required. During the initial phase of the study we were confrontated with the problems encountered by dr. G.H.M. Ten Velden and his colleagues in using heat production as a measure of myocardial work. We are grateful to them showing us their original experimental results, far before they were published. Ten Velden (1982) concluded from his study that the possibility to detect differences in metabolic activity from local heat production seems to be limited due to geometric factors at a certain location (such as wall thickness and irregular wall surface) and by the possible dependency on blood flow direction. It is to be expected that inducement of ischemia results in an even more complex situation by the inhomogeneity of myocardial blood flow.

Regarding these problems, it was concluded that determination of local heat production would give little valid information about myocardial energetics during ischemia in relation to blood flow and metabolism in different layers of the left ventricle.

### 2.2.2 Energy-rich phosphate metabolism

In situ the rate of degradation and resynthesis of ATP is difficult to measure (Gibbs, 1978; Vary et al, 1981). The absolute contents of ATP and creatine phosphate in myocardial tissue only give information about the

availability of these substances at a certain time. This is in agreement with the finding that during normal perfusion, no relation exists between myocardial blood flow and ATP content (Kleinert and Weiss, 1981). Therefore, although most authors found a lower content of ATP and creatine phosphate in the inner layers (Boerth et al, 1969; Dunn and Griggs, 1975; Ichihara and Abiko, 1975; Allison et al, 1978) but not by others (Griggs et al, 1972; Opie, 1976), this cannot be used as evidence for a higher energy demand of these layers.

In the present study, ATP and creatine phosphate contents have been determined to assess the amounts of these substances present under ischemic conditions. Decreased contents of these substances have been mentioned to be related to impaired mechanical activity and irreversible cell damage (Gudbjarnason et al, 1970; Jennings et al, 1978; Hearse, 1979; Reimer et al, 1981). The time course of the breakdown of energy-rich phosphates was also assessed by measuring the release of inorganic phosphate into the local venous blood draining the ischemic area.

## 2.3 Substrate metabolism

### 2.3.1 Global substrate metabolism under normoxic circumstances

Cardiac energy metabolism is highly oxygen-dependent. Even at reduced oxygen supply, oxidative metabolism remains the predominant source (over 90%) of residual ATP production (Neely et al, 1975; Opie, 1976). Myocardial tissue is able to extract almost all oxygen from the blood. Under normoxic conditions, the extraction of oxygen from the arterial content is 60-70% (Van der Vusse et al, 1979; 1981a) which favors the hypothesis that myocardial blood flow is directly related to the oxygen consumption of the blood (Mohrman and Feigl, 1978). During ischemia, up to 90% of the arterial oxygen content is extracted (Van der Vusse et al, 1979).

Of the variety of fuels available to the aerobic heart, the major substrates are NEFA, lactate, glucose and triacylglycerol. Their rates of incorporation and utilization are dependent on their respective concentrations in blood, the availability of hormones (like insulin, catecholamines) and the metabolic rate of oxidative phosphorylation as determined by the mechanical activity of the heart.

Historically, the incorporation and utilization of glucose as a cardiac substrate has received the greatest attention and detailed knowledge of its

regulation is available.

The normal rates of glucose utilization are low and NEFA and lactate are preferentially utilized when present. Uptake of glucose by the myocytes is carrier mediated, not dependent on energy but highly dependent on the presence of insulin.

Uptake of lactate by normoxic myocardium has been described extensively. At high arterial concentrations, lactate may be responsible for up to 87% of total $CO_2$ production or oxygen consumption (Drake et al, 1980; De Jong, 1977a) The mechanism of lactate uptake, however, is poorly understood. Chiong (1973) has communicated a myocardium-arterial blood gradient of lactate under normoxic conditions so that an active transport mechanism in the myocardium may be responsible for the uptake of this substrate. This is in agreement with the findings of Opie and Mansford (1971) in the isolated heart preparation.

NEFA are oxidized in preference to carbohydrates and in aerobic hearts normally account for more than half the substrates utilized for oxidative metabolism. About half of the NEFA taken up from the blood is oleic acid (Rothling and Bing, 1961; Van der Vusse et al, 1982). Serum triacylglycerol fatty acids are a potential source of substrates for myocardial tisue. However, uptake of these fatty acids from the blood is only possible in the presence of lipoprotein lipase on the endothelial cells (Jansen, 1975) an enzyme released by heparin. Also triacylglycerol uptake may be dependent on anesthesia and manipulation of the heart (Van der Vusse et al, 1981a).

Under similar conditions, the sum of the oxygen equivalences of the four substrates mentioned above is lower than the measured oxygen uptake by the heart (Van der Vusse et al, 1981a). This indicates that other substrates are extracted or that endogenous stores of triacylglycerol and/or glycogen are used. This would imply that no steady state is reached under these experimental conditions. However, in chapter 4, it will be shown that under normoxic conditions the myocardial glycogen content does not change within a period of 2 hours. The metabolism of these endogenous substrate stores has been reviewed recently by Van der Vusse and Reneman (1982).

## 2.3.2 Transmural differences in metabolism during normoxia

Because of the supposed higher workload in the inner than in the outer layers of the left ventricular wall, much attention has been payed to metabolic differences between these layers, especially since differences in

workload itself cannot be measured adequately.

Glycogen content has been shown to be higher in the inner than in the outer layers in dog as well as in ox hearts (Jedeikin, 1964; Ichihara and Abiko, 1975; Allison et al, 1977). Carefully performed dissection studies revealed that especially the conductive tissue (i.e. bundle of His and Purkinje fibers) contained large amounts of glycogen (Jedeikin, 1964) which was substantiated by an electronmicroscopic study of Crass and Sterrett (1975).

Triacylglycerol contents have been found to be about 2.5 times higher in the outer than in the middle and inner layers of the left ventricle (Crass and Sterrett, 1975; Jesmok et al, 1978; Van der Vusse et al, 1982). However, since fat is macroscopically visible in the perivascular epicardial regions, the observed transmural gradient of triacylglycerol does not necessarily imply a difference in content of fat in the myocytes of the various myocardial layers.

No information could be found about transmural differences in glucose content. NEFA contents in the inner, middle and outer layers are not significantly different (Van der Vusse et al, 1981b; 1982). Contents of lactate and pyruvate in the inner layers have been found to be 1-1.5 times the contents in the outer layers, the difference being not significant for lactate in 2 out of 5 and for pyruvate in 1 out of 2 studies (Griggs et al, 1972; Dunn and Griggs, 1975; Ichihara and Abiko, 1975; Opie, 1976; Allison et al, 1977). Glucose-6-phosphate has been shown to be 1.5 times higher in the inner than in the outer layers (Ichihara and Abiko, 1975). The higher glycolytic activity in the inner layers as suggested by these results is in agreement with the findings of Lundsgaard-Hansen et al (1967) who reported higher contents of 4 glycolytic enzymes (including lactate hydrogenase) in the inner layers. However, transmural gradients of the latter enzyme were not found in more recent investigations (Dowell, 1978; Visser et al, 1981). One should realize that these data only refer to the content of substances and enzymes at a certain moment and do not give direct information about the actual glycolytic flux in vivo.

Oxygen extraction in the various layers has been measured by microspectrophotometric observations of small regional arteries and veins. Under normoxic conditions, oxygen extraction was higher in the inner than in the outer layers. Multiplication of these values with those of regional myocardial blood flow resulted in 1.3 and 1.6 times higher oxygen uptake values in the inner layers than in the outer layers (Holtz et al, 1977; Weiss et al, 1978).

These values might be an overestimation of the actual transmural gradient in oxygen uptake since the employed technique for the measurement of regional myocardial blood flow in these studies (e.g. radioactive microspheres) likely overestimates blood flow in the inner layers (section 3.4.2). These results imply that the energy demand of the inner layers exceeds that of the outer layers but not as much as has been suggested by calculation of this demand from measured intramyocardial pressure (Marzilli et al, 1980; 1981). In fact, the differences in oxygen consumption between the inner and outer layers might be small, which is in keeping with the predicted equal workload for these layers from a mathematical model (Arts, 1978).

## 2.3.3 Substrate metabolism during ischemia

A decrease in blood flow causes oxygen extraction to increase up to 90% of the arterial content (Van der Vusse et al, 1979). Under ischemic conditions, glycolysis is stimulated by restrictions in coronary flow (Neely et al, 1975) due to the activation of hexokinase and phosphofructokinase. The increased transport of glucose, as found during anoxia (Morgan et al, 1959) might also play a role during ischemia. Anaerobic glycolysis causes a release of lactate during ischemia, in contrast to lactate uptake during normoxia. Lactate extraction or production have been used to assess the presence or absence of myocardial ischemia in humans (Williamsom, 1966). However, this assessment is complicated by the facts that lactate extraction is dependent on arterial NEFA concentrations (Gertz et al, 1980) and lactate release from ischemic tissue is dependent on the time interval after onset of ischemia and is not linearly related to the degree and the amount of ischemia (Apstein et al, 1979).

Beside the increase in glucose uptake from the blood, glycogenolysis is stimulated which may ultimately lead to depletion of these stores after prolonged ischemia (reviewed by Van der Vusse and Reneman, 1982).

Fatty acid oxidation is greatly depressed under ischemic conditions, mainly due to inhibition of beta-oxidation which is demonstrated by mitochondrial accumulation of long-chain acyl-CoA and cytosolic acylcarnitine (Idell-Wenger et al, 1978). Besides, NEFA uptake from the blood is continued during ischemia (Opie et al, 1973; Van der Vusse et al, 1982). The NEFA taken up in excess of residual combustion can be accumulated as NEFA or as triacylglycerol. Accumulation of the latter substance has been reported (Evans, 1964). Controversely other investigators found increased release of NEFA and glyce-

rol from endogeneous triacylglycerol stores (Opie et al, 1973; Vik-Mo et al, 1979).

Accumulation of NEFA in the ischemic myocardium is shown by Weishaar et al (1977, 1979) and Van der Vusse et al (1982), but not by Regan et al (1972) and Haider et al (1977). Increased myocardial NEFA contents are of interest because several harmful effects have been ascribed to elevated cellular NEFA concentrations. In vitro studies showed that NEFA concentrations, in the same order of magnitude as found during ischemia (Van der Vusse et al, 1982), inhibit glycolytic enzymes and uncouple mitochondrial ATP production, but that addition of albumin, simulating specific NEFA binding proteins (section 2.1.2), abolished or greatly depressed these deleterious effects (Borst et al, 1962; Randle et al, 1964; Lea and Weber, 1968; Ramadoss et al, 1976).

During ischemia, increased acyl-CoA and acylcainitine concentrations may have a detergent action on mitochondrial membranes (Feuvray and Plouet, 1981). Also elevated acyl-CoA and acylcarnitine seemed to be related to early mechanical failure after onset of ischemia (Pitts et al, 1978; Feuvray et al, 1979). Recent reviews on this subject were published by Katz and Messineo (1981), Liedtke (1981), Vik-Mo and Mjøs (1981) and Van der Vusse (1982).

It has to be questioned whether the NEFA accumulation, as shown by Van der Vusse et al (1982), has deleterious effects on the ischemic myocardium. There are several arguments which make a positive answer doubtful.

Comparisons of the harmful NEFA concentrations in vitro and the NEFA contents as measured in myocardial biopsies should be carefully made, since the biopsies contain 5-10% blood in which the NEFA content is considerably higher than in the tissue itself. Normoxic NEFA concentrations in myocytes have been calculated to be 10 $nmol.g^{-1}$ as compared to 30 $nmol.g^{-1}$ in biopsies (Van der Vusse et al, 1981b, 1982).

Increased cellular concentrations of linoleic and arachidonic acid (Van der Vusse et al, 1982, during ischemia and chapter 7 of this thesis) may result in an increased biosynthesis of prostaglandins in ischemic myocardial tissue. Enhanced prostaglandin $F_{2\alpha}$ synthesis and increased release of this substance from regional ischemic dog hearts have been reported (Berger et al, 1976). Protective effects of this prostaglandin have been described in animals after coronary artery occlusion (Goldfarb and Glenn, 1974).

In the present study the time course of NEFA accumulation has been compared with that of the changes in blood flow, metabolism and mechanical performance in the ischemic myocardium (chapter 7), which may indicate,

whether the increase in myocardial NEFA content preceeds inhibition of pro-
posed affected processes.

## 2.4 Myocardial blood flow

### 2.4.1 Myocardial blood flow and its transmural distribution

The myocardium is supplied with blood through epicardial coronary arteries
that divide on the surface and send off tributaries at approximately right
angles into the myocardium. There are indications that the epicardial and
middle layers are perfused by other branches than the endocardial layers
(Estes et al, 1966). The endocardial branches subdivide into anastomosing
arcades, forming a large subendocardial plexus.

Under physiological conditions regulation of myocardial blood flow is
mainly achieved by modifying the smooth muscle tone, and hence the diameter,
of the arterioles. Although coronary vessels are innervated by sympathetic
(Feigl, 1975a) and parasympathetic nerves (Feigl, 1975b), it is likely that
the regulation of myocardial blood flow is dominated by myocardial metabolism
because coronary blood flow is directly related to the oxygen consumption of
the myocardium (Allella et al, 1955; Mohrman and Feigl, 1978). Such factors
as $P_{O2}$, $P_{CO2}$, $H^+$, $K^+$, adenosine, lactate, osmolarity, prostaglandins and
serotonine have been proposed as candidates for linking metabolic activity
and arteriolar tone, but the best documented hypothesis for this feedback
control is that adenosine is the mediator substance (Berne, 1963; Rubio and
Berne, 1969). It is likely that in autoregulation of coronary blood flow,
defined as the tendency to maintain blood flow constant despite changes in
perfusion pressure, changes in pressure across the vessel wall play a part in
the changes in arteriolar diameter. In various tissues, after all, a decrease
in this pressure is followed by dilation of the arterioles (Bouskela and
Wiederhielm, 1979; Burrows and Johnson, 1981), even when venous pressure is
elevated simultaneously (Reneman et al, 1980), while an increase in pressure
across the vessel wall induces arteriolar constriction (Boushkela and Wieder-
helm, 1979).

Myocardial blood flow is impeded by ventricular contraction. This has been
explained by the formation of vascular water falls (Downey and Kirk, 1975) or
by the existence of an intramyocardial pump (Arts, 1978; Spaan et al, 1981).
The reduction of myocardial blood flow by extravascular compression is not
uniform across the ventricular wall and is determined by intra-myocardial

pressure ($p_{im}$). This quantity can be defined as the pressure in the weak incompressible material surrounding the contractile fiber structure. None of the methods developed to measure $p_{im}$ is reliable (Arts and Reneman, 1977) because of the induced damage and/or disalignment of the contractile fibers. The transmural course of $p_{im}$ can be predicted from a model of the mechanics of the left ventricle in which myocardial material is assumed to be anisotropic and rotation of the apex relative to the base around the axis of the ventricle is allowed (Arts and Reneman, 1977; Arts et al, 1979). In the left ventricle, $p_{im}$ is higher in systole than in diastole, and higher in the inner than in the outer layers. In the inner most layers, $p_{im}$ equals the pressure in the left ventricular cavity and in the outer most layers, it is zero. The equality of endocardial $p_{im}$ to left ventricular cavity pressure is a boundary condition. The described pressure relationships imply that blood flow to the wall of the left ventricle mainly occurs during diastole, when the difference between aortic pressure and $p_{im}$ is greatest. In systole, blood flows mainly to the outer layers. Since mean transmural blood flow in the left ventricle is approximately uniform (Buckberg et al, 1972) (chapter 4), the gradient in the distribution of flow during systole must be compensated by a similar but reversed gradient during diastole. The latter idea is supported by the finding that the conductance is higher in the subendocardial than in the subepicardial vessels (Rouleau et al, 1979; Wuesten et al, 1977), but in disagreement with the finding that left ventricular transmural blood flow is uniform during diastole (Hess and Bache, 1979).

Recently, it has been suggested that diastolic $p_{im}$ might be higher than generally accepted and even exceed diastolic pressure in the left ventricular cavity (Hoffman, 1978; Rouleau et al, 1979). This idea was derived from the finding that extrapolation of the diastolic coronary pressure-flow relation down to zero flow resulted in an intercept on the pressure axis at pressures between 2.7 and 7.2 kPa. This zero flow pressure, however, does not necessarily relate to $p_{im}$ alone. In skeletal muscle experiments, capillary blood flow was found to stop at a perfusion pressure (arterial minus venous pressure) of about 3.3 kPa in the presence of an intramuscular pressure of approximately 0.75 kPa (Reneman et al, 1980), indicating that other (rheological) factors may be involved in inducing this zero-flow pressure.

Decreased blood flow due to partial or total coronary artery occlusion and reduced coronary artery pressure results in relative underperfusion of the subendocardial layers (Domenech et al, 1969; Rivas et al, 1976; Bishop et al,

1976). Even when blood flow in the inner layers was severely affected, vessels in the outer layers proved to be not yet fully dilated (Gould et al, 1975). This underperfusion of the inner layers of the left ventricular wall is caused by the higher $p_{im}$ in these layers (Hoffman, 1979) and is the major cause of the greater susceptability of these layers for impedement of coronary flow, since a higher energy demand of the inner layers is disputable (see above).

## 2.4.2 Measurement of regional myocardial blood flow

Myocardial blood flow can be determined with such techniques as: electromagnetic and Doppler, differential pressure, continuous thermodilution, local heat and gas clearance ($H_2$, $N_2O$, He, $Xe^{133}$, $Kr^{85}$), diffusable tracer ($K^{43}$, $Rb^{81}$, $Cr^{131}$, $Th^{201}$, I-antipyrine) and microspheres.

With regard to the aim of the present study (chapter 1), blood flow has to be measured: (a) in different layers of the myocardium, (b) at various time intervals within each experiment, (c) in the entire free wall of the left ventricle, enabling localization of the ischemic area in the myocardium, and (d) with acceptable accuracy, to make the comparison between myocardial blood flow and local mechanical performance and metabolism meaningful.

Most flow measuring methods are unsuitable because they faile to give information about the transmural distribution of myocardial blood flow. Unlabeled microspheres (as determined light microscopically) can be used only once in each experiment. Using local inert gasses or heat clearance, the ischemic area cannot be localized properly. The use of diffusable tracers to detect myocardial blood flow in more than one area requires elaborate equipment. With diffusable tracers, this localization is possible when a scanning camera is used. This method, however, is very expensive, more elaborate and hardly reaches the resolution of the microsphere method. With the latter technique, a satisfactory resolution in transmural direction as well as localization of the ischemic area can be reached by adaptation of the sample size. Besides, measurements can be performed several times in each experiment and the method has a good accuracy if the number of microspheres per sample is adequate (section 3.4). Moreover, application of the technique is relatively simple, though its limitations should be considered.

## 3. MATERIALS AND METHODS

### 3.1  Experimental set-up

#### 3.1.1 Choice of the animal model

Measurement of mechanical performance, blood flow and metabolism selectively in the perfusion area of one coronary artery requires hearts of at least 150 gram, determines the minimal size of the experimental animal. For these types of experiments, one generally uses the pig or the dog. The former species is preferred by some investigators because of the presumed similarity between pig and human heart (De Jong and Goldstein, 1974; Liedtke et al, 1975; Verdouw et al, 1978, 1979). Indeed both man and pig have a dominant right coronary artery or left anterior interventricular coronary artery. However, the number and type of collaterals (epi- or endocardial, and size) in the human heart is about in between those of the dog and the pig heart (Schaper, 1971a,b; Gregg, 1974). The use of the dog was preferred in the present study because (a) the dog is the most widely used experimental animal in heart research allowing the comparison with results obtained by other investigators; (b) pigs suffer from a high incidence of cardiac arrhythmias and ventricular fibrillation, especially during ischemia (Verdouw et al, 1978). This may be beneficial for the investigation of anti-arrhythmic drugs but it is undesired in the present study; (c) the dominance of the left circumflex coronary artery in the dog is not crucial in interpreting the results of the present investigation.

Heart function and metabolism of the heart should be studied in a condition similar to the normal situation. Therefore, the in situ preparation is superior to any isolated heart preparation. Of course an awake closed-chest animal is the best model, because anesthetics and opened chest influence the performance of the heart. However, the application of so many techniques at the same time requires the heart to be in reach. For that reason, an open-chest preparation was used in which accurate limitation of coronary blood flow was possible (section 3.1.4), which made the use of a separate coronary artery perfusion line (Liedtke et al, 1978; Weintraub et al, 1981) super-fluant.

#### 3.1.2 Anesthesia

The experiments were performed on mongrel dogs of either sex and unknown

age, ranging in weight from 18 - 60 kg. The animals were premedicated with Hypnorm (1 ml.kg$^{-1}$ body weight i.m.) as described by Marsboom et al (1964). One ml Hypnorm contains 10 mg fluanisone and 0.315 mg fentanyl citrate. Anesthesia was induced with sodium pentobarbital (10 mg.kg$^{-1}$ body weight i.v.) and after endotracheal intubation, was maintained with oxygen/nitrous oxide and a continuous infusion of sodium pentobarbital (2 mg.kg$^{-1}$.hour$^{-1}$ i.v.). Ventilation was kept constant during the experiments with a positive pressure respirator (Pulmonat).

Decrease of body temperature of the animal was prevented by heating and humidifying the inhalated air with a Draeger air humidifier as well as by a heating matrass, placed underneath the animal.

During thoracotomy, succinylcholine (2 mg.kg$^{-1}$ i.m.) was injected to prevent muscle movement caused by electrocoagulation. The chest was opened through the left fifth intercostal space and the pericardium was incised over the antero-lateral aspect of the heart.

### 3.1.3 Instrumentation

ECG was recorded from the limb leads. Signals were amplified by a Schwarzer EC 553 ECG-amplifier. Aortic pressure was measured through a polyethylene catheter, connected to an external pressure transducer (section 3.2.2), which was placed in the aortic arch. Left ventricular pressure ($p_{lv}$) was measured with a Millar catheter-tip micromanometer, inserted through the left brachial artery (section 3.2.2). The rate of change of left ventricular pressure and left ventricular end-diastolic pressure were also determined (section 3.2.2). A small polyethylene catheter (P.E.50, Clay Adams) was placed in a small side branch of the left anterior interventricular coronary artery for determination of the arterial pressure (Van der Meer and Reneman, 1972) distal to the partial occlusion (or: stenosis) (fig 3.1, section 3.1.4). An electromagnetic flowprobe was placed around the ascending aorta for the measurement of phasic aortic flow (section 3.2.3). Cardiac output was measured by thermodilution using a digital analyzing technique (Snoeckx et al, 1976). Through a branch of the left jugular vein, a 7F Swan-Ganz balloon guided thermistor catheter was placed in the pulmonary artery. For the injection of saline at room temperature, a separate injection catheter (8F NIH, USCI) was inserted through another side branch of the jugular vein and advanced till the transition of the superior caval vein into the right atrium. Both catheters were placed in position under fluoroscopic control.

Fig 3.1   Experimental set-up. In the open-chest preparation, an occluder was placed around the left anterior interventricular coronary artery (LAICA). During ischemia, post-stenotic coronary artery pressure was kept constant using an autoregulating feedback system. Coronary artery pressure was measured through a catheter inserted into a small side branch of the LAICA, distal to the site of stenosis. Local venous blood was sampled through a catheter inserted into the left anterior interventricular coronary vein. Regional epicardial surface deformation was measured with three coils, sucked on the epicardium in the LAICA perfusion area. For the determination of substances in the perfusion area of the LAICA and the left circum-flex coronary artery (LCCA), small biopsies were taken for ATP, creatine phosphate and glycogen determination and large biopsies for fatty acid determination.

A polyethylene catheter (P.E.60, Clay Adams) was inserted into the left anterior interventricular vein with a Seldinger technique to obtain venous blood from the area distal to the site of stenosis (fig 3.1).

To inject radioactive microspheres for the assessment of regional myocardial blood flow (section 3.4), a silastic catheter was placed in the left atrium. A polyethylene catheter was inserted into the right brachial artery to obtain blood for the arterial reference sample, required for the calculation of regional blood flow (section 3.4).

For the measurement of epicardial deformation according to Arts and Reneman (1980) (section 3.3), three coils were attached to the epicardium (fig 3.1). The field-generating coil was placed on the free wall of the left ventricle, in the perfusion area of the left anterior interventricular coronary artery (LAICA). The two sensor coils were placed in the same area, both at a distance of about 15 mm from the magnetic field-generating coil. The three coils were placed so that they formed an approximately rightangled triangle with the field generating coil at the rightangled corner and the sides containing the right angle, parallel to the circumferential and base-to-apex direction, respectively. In this way, shortening in circumferential and base-to-apex direction could be measured as well as shear deformation (section 3.3). The coils were attached to the epicardium by suction (underpressure approximately -25 kPa).

All hemodynamic variables as well as those from the measurement of epicardial deformation were recorded continuously on a multichannel Schwarzer recorder (upper frequency response 280 Hz, -3dB) at a speed of 1.25 mm.s$^{-1}$ and at the sample times at speeds of 50 and 200 mm.s$^{-1}$.

### 3.1.4 Stenosis system

Partial occlusion (stenosis) of the LAICA was induced with an inflatable cuff which was placed around this artery just distal to the diagonal branch (fig 3.1).

The cuff was through silastic tubing connected to a micrometer. The whole system was filled with distilled water so that the cuff could be inflated carefully until the desired degree of stenosis was obtained. This degree of stenosis could be maintained during the experimental period, using a servo motorpump with an autoregulating feedback system controlled by the mean coronary artery pressure ($p_{cor}$) (fig 3.1).

In the present study, mean $p_{cor}$ as measured distal to the stenosis in a

small side branch of the LAICA rather than the reduction of mean flow in this artery was used to estimate the degree of ischemia. Under these circumstances, the determination of pressure is easier and more accurate (Van der Meer and Reneman, 1972). Moreover, during ischemia flow in the coronary microvasculature is related to the perfusion pressure in that bed and less related to arterial inflow because of an unknown amount of collateral inflow. At last, the servocontrol corrects for possible movements of the cuff along the vessel, which may cause changes in the degree of stenosis.

Despite the similarity of mean $p_{cor}$ values distal to the stenosis, interindividual variations were seen in regional blood flow, mechanical performance and metabolism during ischemia. This may be due to interindividual differences in coronary resistance, collateral circulation or aortic pressure. The latter factor increases myocardial energy requirement, so at a certain reduced flowlevel, ischemia will be more pronounced at higher aortic pressure.

The occlusion of the side branch caused a small area of underperfusion distal to the catheter, as could be shown by determination of regional myocardial blood flow (section 3.4). However, this occlusion had no deleterious effect on the experiments. General cardiac function was not influenced by this procedure and cardiac arrhythmias due to the occlusion were not observed. For the calculation of total regional blood flow in the ischemic zone, this area of underperfusion was excluded because of possible artefacts due to the pre-existing ischemic tissue.

In the study dealing with total coronary artery occlusion (chapter 5) the cuff was fully inflated, whereafter the tubing was closed with a stopcock.

### 3.1.5 Technique of blood sampling

Arterial blood samples were collected through the catheter used for aortic pressure measurements. Local venous blood samples were obtained through the catheter in the left anterior interventricular coronary vein (fig. 3.1). Blood (0.6 ml) was carefully sucked into a syringe filled with 0.1 ml heparin (5000 $I.U.ml^{-1}$) and used for determination of blood gases and blood pH which were determined immediately after sampling (section 3.5). To reduce gas exchange with the environment between sampling and determination, the syringe was locked by stainless steel syringe caps. From the syringe with blood for the blood gas determination, 40 $\mu l$ was taken with a heparinized capillary and entered into a hemoxymeter to determine oxygen content of the blood (section

3.5.3). Another 4-6 ml blood (for other chemical determinations) were rapidly centrifuged for 1 min in a Eppendorf centrifuge. The supernatants of these samples were quickly frozen and stored at $-80^{o}C$ for biochemical analysis. The time from sampling to freezing varied in most cases from 2 to 5 min.

When the local venous catheter was not closed by a stopcock, in most cases blood dripped out of the catheter, although too slow for sampling purposes. Therefore, blood was sucked using 1 or 2 ml syringes. A critique to this technique is that it cannot be excluded that blood is also sampled from adjacent vascular beds via venous collaterals. Especially during ischemia, this could be a problem in interpreting the results because of admixture of blood from normoxic tissue with that from ischemic tissue. However, measurement of regional myocardial blood flow, revealed that in an ischemic zone of about 20 g, which is a common figure in our model, a flow of 4 $ml.min^{-1}$ is present. When venous blood samples are drawn, 4-6 ml of blood are withdrawn in the course of a few minutes. This implies that the volume of venous blood sampled over a certain period does not exceed the arterial inflow. Moreover, in the present study the ischemic area is relatively large as compared with experiments of Owen et al. (1970), who created ischemic areas of 7-8 g. Therefore, in our study, the contribution of blood from the borders of the ischemic area or from outside this area will be small as compared with the study of Owen and co-investigators (1970). If admixture through venous collaterals occurs, changes due to ischemia will be underestimated. It should be emphasized that it is not clear whether the venous blood samples reflect the average of transmural venous blood or that mainly blood is obtained from one particular layer.

### 3.1.6 Techniques of tissue sampling

In this study two types of samples were taken from the left ventricular myocardium: samples for determination of the content of: (a) ATP, creatine phosphate, and glycogen; (b) fatty acids.
a. Biopsies for the determination of energy-rich phosphates and glycogen were taken with a pressured air driven drillbore (Schrader) on which drills were mounted with a length of 4 cm and an inner diameter of 4 mm. The wet weight of the biopsies ranged from 50 to 70 mg. Because of the deleterious effects of tissue sampling on regional blood flow and mechanics, shown in chapter 4, it was decided to stop the experiment after taking two biopsies. At first a transmural biopsy was taken from the area perfused by the

LAICA. An assistant put his finger on the hole to prevent bleeding as much as possible. Subsequently, within 1-2 min, a biopsy was taken from the left circumflex coronary artery (LCCA) perfusion area (fig 3.1). After the drill was withdrawn from the heart, it was immersed in liquid nitrogen while still mounted on the bore. The time delay between taking the biopsy and the immersion in liquid nitrogen was 2-3 s. After cooling of the drill, it was removed from the bore and put into a cold resistant polypro-pylene tube filled with liquid nitrogen and subsequently stored in a Dewar container filled with the same fluid. After termination of the experiment, the tissue containing drills were stored at $-80^{\circ}C$. After freeze drying these samples were divided into subendo, meso and subepicardial tissue samples.

b. Biopsies for the determination of myocardial fatty acid content were taken from the left ventricular free wall with an electrically driven drillbore, on which drills were mounted with a length of 2 cm and a inner diameter of 1 cm.. The first tissue sample was taken from the LAICA perfusion area. Subsequently, two biopsies were taken from the area perfused by the LCCA and at last another biopsy from the LAICA perfusion area was taken. The whole biopsy procedure took about 5 min. In most cases, ventricular fibri-llation occurred after taking the second biopsy. No significant differen-ces have been observed between biopsies taken before and within 5 min after onset of ventricular fibrillation. The wet weight of the biopsies ranged from 500 to 900 mg. Adhering blood was removed with an ice-cold physiological saline solution. Then the biopsies were divided into subepi, meso and subendocardial samples. Subsequently, the samples were frozen between the tongues of an aluminum clamp which was cooled in liquid nitro-gen. The whole procedure from taking the biopsy till freeze-clamping of the tissue took less than 15 seconds. The frozen tissue samples were stored at $-80^{\circ}C$.

## 3.1.7 Anti-coagulation

Administration of heparin was deliberately avoided because of its influ-ence on myocardial metabolism. Heparin, after all, releases lipoprotein lipase from the endothelial wall, thereby preventing the myocardium to use triacylglycerol as a substrate (Jansen, 1975). Besides, the release of lipo-protein lipase increases arterial non-esterified fatty acids (NEFA) concen-trations (Riemersma et al 1977b; Van der Vusse et al, 1980) which may affect

the relative contribution of NEFA combustion to myocardial substrate supply (Kjekhus and Mjøs, 1972; Dagenais and Jalbert, 1977). Moreover, the use of heparin causes a more rapid in vitro lipolysis in serum, which gives rise to artefacts in the measurement of blood NEFA concentrations (Giacomini et al, 1980). The effect of elevating the arterial NEFA concentrations during ischemia by administration of heparin, intralipid or both substances is described and discussed in chapter 8.

Avoiding the administration of heparin had also the advantage that less bleeding occurred from surgical wounds like the edge of the thorax and from the sites of insertion of the catheters.

Catheters were kept patent with a continuous infusion of saline. The catheters for measurement of aortic and coronary artery pressure and for microsphere injection were connected to a Sorenson Intraflo continuous infusion system with an infusion rate of 6 $ml.h^{-1}$ (Sorenson Research Company, Salt Lake City, USA). The thermodilution injection catheter, the local venous catheter and the arterial reference sample catheter were connected to syringes mounted on Harvard infusion/withdrawal pumps (infusion at a rate of 10-12 $ml.h^{-1}$). Although no saline infusion was performed through the lumen of the catheter tip micromanometer, no influence of the deletion of heparin was observed on the pressure measurement with this micromanometer: an identical signal was obtained with an external Ailtech transducer measuring through the lumen of the micromanometer.

## 3.2 Hemodynamic measurements

### 3.2.1 Introduction

Measurement of general hemodynamic parameters like ECG and aortic and left ventricular pressure were performed because they provide information about the cardiovascular condition of the experimental animal. Moreover, the separately recorded end-diastolic left ventricular pressure and the maximal first derivative of the left ventricular pressure ($dp_{lv}/dtmax$) are sensitive parameters to assess the condition of the left ventricle. Measurement of left ventricular pressure using a catheter-tip micromanometer provides a signal with minimal time delay (<1 ms) or reflections due to the large pressure variations, as is the case with waterfilled catheters. The coronary artery pressure distal to the stenosis was determined to serve as a measure for the degree of ischemia (section 3.1.4). Electromagnetic measurement of aortic

flow was used to describe the ejection phase of left ventricular contraction (essential for the interpretation of the results on the assessment of regional mechanical performance).

Electromagnetic measurement of flow in the LAICA was not performed because in the majority of the experiments one or more side branches of this artery had to be ligated to be able to implant both the occluder cuff and the flow probe. Moreover, more detailed information about myocardial blood flow can be obtained with the microsphere method (section 3.4).

Signals of left ventricular pressure, aortic and coronary artery pressure as well as phasic aorta flow and the ECG were contiuously visualized on a Knott GS-8 monitor.

## 3.2.2 Blood pressures and derived parameters

The external Ailtech pressure transducer in series with the aortic sample catheter had a frequency response of 35Hz (-3dB). In series with the catheter in the side branch of the LAICA, the frequency response was 15Hz (-3dB).

The catheter-tip micromanometer (7F Millar, PC470) for the measurement of left ventricular pressure ($p_{lv}$) had a frequency response of 0-20 kHz. The maximal first derivative of left ventricular pressure ($dp_{lv}/dtmax$) was determined with an analog differentiator as described by Schaper et al (1965). End-diastolic left ventricular pressure ($p_{lved}$) was separately recorded by clipping the $p_{lv}$ at 200 mV (2.7 kPa) and subsequently amplifying the signal on the recorder.

The pressure transducers were calibrated before each experiment. The output voltage for 13.3 kPa (100 mmHg) was determined using a mercury column. An electrical output signal and the signals on the recorder and the monitor were thus calibrated, barometric pressure serving as zero reference. A pressure of 13.3 kPa gave an output signal of 1 Volt. On the recorder 13.3 kPa was equal to 20 mm, except for the $p_{lved}$, where 20 mm represented 2.7 kPa.

At each sample time, the zero and electrical full scale calibration of the external transducers were checked on the monitor and the recorder. By an external pressure transducer, measuring the pressure through the lumen of the Millar catheter-tip micromanometer, the zero shift of this manometer was checked and was corrected if necessary. The gain of the Millar transducer did not change noteworthy during the experiment as was checked by comparing the amplitude of the signals obtained from the Ailtech and Millar transducers on the monitor. In this procedure the variation in gain of the Ailtech transdu-

cer, though small, could not be checked.

### 3.2.3 Blood flow (electromagnetically measured)

Phasic aortic flow was measured with an electromagnetic flow probe (Skalar). The probes were connected to a sine wave electromagnetic flowmeter with a carrier frequency of 600 Hz (Transflow 600). The upper frequency response of the flowmeter was 100 Hz (-3 dB).

Zero drift of the flow probe was checked by comparing the electrical zero with the end-diastolic level of the phasic flow signal on the monitor. Because phasic aortic flow was used to assess the start and duration of the ejection phase and no quantitative flow measurement was required, full scale calibration was not performed.

To determine the start of the ejection phase accurately a time delay has to be taken into account. Time delay firstly occurs due to the distance between the valve and the flow probe. With a maximal distance of 5 cm and assuming a pressure pulse wave velocity of 4 $m.s^{-1}$, this delay will be no more than 12 ms. Additional delay (electronic) is induced by filtering in the flowmeter, which was in our case about 3 ms. Hence the total delay between the start of the ejection phase and its assessment from the electromagnetic flowmeter reading will be maximally 15 ms, for which has been corrected.

### 3.2.4 Cardiac output

#### 3.2.4.1 Introduction

Several methods for the determination of cardiac output could have been used in the present study: electromagnetic aortic flow measurement, the radioactive microsphere method or the indicator dilution techniques. The first technique was not employed because in vivo full scale calibration of the flowprobes is problematic and coronary flow is systematically missed. The indicator dilution technique was preferred over the radioactive microsphere method because the former can be performed almost unlimited whereas use of the latter method, in combination with the measurement of regional myocardial blood flow, is limited by the single use of each isotope. Temperature was employed as indicator because of its easy application and low costs of the set-up.

3.2.4.2 Instrumentation

Cardiac output was measured by thermodilution using a Swan-Ganz balloon guided thermistor catheter (Edwards) and a 8F NIH injection catheter (USCI). This method has been described previously in detail by Snoeckx et al (1976). The large injection catheter enabled the injection of a relatively large volume of saline within a short period of time, which enlarges the obtained signal and thereby improving the accuracy of the method. Before injection of saline, blood from the animal was sucked into the catheter to prevent cold dissipation from the catheter. After a steady baseline registration (see below), 10 ml of saline at room temperature were injected manually as fast as possible. Immediately after the syringe was emptied the catheter was filled again with blood, thereby reducing the non-exponential character of the last part of the thermodilution curve due to cold dissipation from the catheter after injection (Vliers et al, 1973).

The catheter was connected to a thermodilution amplifier of our own design. The resistance in the Wheatstone bridge of this apparatus was adjusted to the resistance of the catheter (about 5 kOhm). The temperature measured was shown on a small digital display, allowing the continuous visualization of body temperature.

Thermodilution curves were registrated on a Linseis series 2000 recorder at a speed of 50 $mm.s^{-1}$. The sensitivity of the recording system was calibrated after the experiments with waterbaths of different temperatures and an independent thermometer.

3.2.4.3 Calculation of cardiac output

The area under the curve was measured by integration using a Digital MINC II minicomputer. Points of the curve were stored on file using a Hewlett Packard Digitizer (9874 A), at a sampling rate of 20 Hz. To calculate cardiac output the TERDIL program was used. In this procedure at first the data points were made equidistant. Subsequently a Fourier analysis was performed according to which high-frequency noise was filtered (>20 dB). After backward transformation, the surface of the resultant curve was determined as follows. The onset of the curve was the segment with the highest first derivative of the upstroke (dp/dt). From the points entered before this segment the baseline was calculated. From the onset of the curve to its maximum ($y_{max}$) and from $y_{max}$ to 0.7 x $y_{max}$, the surface under the curve was directly measured. Since the last part of the curve shows aberrations due to recirculating cold

(Snoeckx et al, 1976, Vliers et al, 1973), this part of the curve was assumed to be exponential (Snoeckx et al, 1976). The equation of this exponential curve was determined using the points between 0.7 and 0.3 x $y_{max}$, and extrapolating the curve to zero. Thereafter the surface under this part of the curve was measured and added to the two parts integrated before.

Cardiac output (CO) was calculated according to Snoeckx et al (1976):

$$CO = K.\frac{V_i \cdot (T_b - T_i)}{S \cdot k_1 \cdot k_2} .k_3 \quad \text{and} \quad K = \frac{s_i \cdot g_i}{s_b \cdot g_b}$$

where $s_i$ = specific heat injectate (100 kcal $kg^{-1}$ $^oC^{-1}$), $g_i$ = specific weight injectate (0.99 x $10^3$ kg $m^{-3}$), $s_b$ = specific heat blood (0.87 kcal $kg^{-1}$ $^oC^{-1}$), $g_b$ = specific weight blood (1.05 x $10^3$ kg $m^{-3}$), $V_i$ = volume injectate ($m^{-3}$), $T_b$ = temperature blood ($^oC$), $T_i$ = temperature injectate ($^oC$),S = surface under the curve ($m^2$), $k_1$ = velocity recorder (s $m^{-1}$, in our experiments 1.2 x $10^{-2}$), $k_2$ = sensitivity recorder ($^oC$ $m^{-1}$), $k_3$ = correction factor from ml $s^{-1}$ to l $min^{-1}$ (=0.06).


3.2.4.4 Accuracy of the cardiac output determination

Because each dog served as its own control, we will only discuss the variations between two measurements in the same animal. The intraassay coefficient of variation in the determination of cardiac output by thermodilution can origin from one of the following factors:

a. variations in the injected volume. Repeated measurements of this volume revealed a mean variation of 0.6%.
b. variations in the difference between injectate and blood temperature. Because both temperatures were measured with an accuracy of $0.1^oC$ and the difference between these temperatures was usually 15 $^oC$, unnoticed variations in the temperature will not account for more than 0.6% variation.
c. variation in the determination of the surface area under the curve. This variation is caused by deviations of the digitizer pen from the recorded curve. Repeated measurements of one curve showed that this variation was 1.2%.

Measurement of two to four curves recorded within 1-2 min from each other during a stable hemodynamic situation showed variations of 2.8 to 9.9%, with

a mean of 6.7%. The difference between this variation and the sum of the variations mentioned in (a) through (c) being 1.4%, might be caused by base-line fluctuations, inaccuracies in determining the baseline determination and difference in the shape of the curve, causing variation in exponential extra-polation of the last part of the curve.

## 3.3 Regional mechanical function

### 3.3.1 Introduction

Keeping in mind the aim of this study, a technique for the assessment of regional mechanical performance in different layers of the left ventricular wall is required. In chapter, it has been emphasized that several of the techniques available to determine this performance in the various layers are subject to artefacts or are less appropriate for this study. In the present study, we have used local epicardial deformation to estimate regional mecha-nical function. This deformation can be reliably and relatively easily mea-sured in the open-chest dog preparation. Moreover, this technique does not only give information about the decrease of the epicardial surface area during ejection, which is directly related to the local contribution to the decrease of left ventricular cavity volume, but it gives also information about the transmural course of fiber shortening by assessment of a shear com-ponent beside circumferential strain and base-to-apex shortening.

The technique of measuring epicardial deformation is described in detail by Arts and Reneman (1980). Therefore, the principles and instrumentation are only briefly mentioned below.

To investigate the reliability of the assessment of the transmural course of fiber shortening from epicardial deformation, a technique was developed to measure strains in the epicardium as well as in the endocardium (section 3.3.3).

### 3.3.2 Epicardial deformation

#### 3.3.2.1 Principles and instrumentation

Assume a rectangle OABC on the epicardial surface prior to deformation. During contraction the rectangle OABC (fig 3.2) deforms to a parallelogram OA'B'C'. Define O as the origin, OA and OA' along the c-axis (circumferential direction) and OC along the z-axis (base-to-apex direction, which is perpendi

# THEORETICAL MODEL



$$e_c = \ln(OA'/OA)$$
$$e_z = \ln(OC' \cdot \cos\gamma / OC)$$

Fig 3.2
Rectangle OABC deforms to parallelogram OA'B'C'. Deformation is defined by natural strain $e_c$ along OA, natural strain $e_z$ along OC and shear angle $\gamma$.

# PRACTICAL APPROACH



$$e_c = -\frac{1}{4}\ln(V_C/V_{C,ref})$$
$$e_z \approx -\frac{1}{4}\ln(V_z/V_{z,ref})$$
$$\gamma = 90° - \text{phase shift}(V_C/V_z)$$

Fig 3.3
Measurement of the deformation parameters. A magnetic field rotates around the magnetic field-generating coil (MFGC) and induces voltages $V_C$ and $V_z$ in sensor coils $SC_C$ and $SC_z$, respectively. The deformation parameters are determined from the amplitudes of and the phase difference between $V_C$ and $V_z$.

cular to the c-axis). Natural tangential strain ($e_c$), natural axial strain ($e_z$), and shear angle $\gamma$ are defined as indicated in fig 3.2.

In the experimental set-up three coils are attached to the epicardial surface, one magnetic field-generating coil (MFGC) at the origin O and two sensor coils at the positions A and C. The field-generating coil is supplied by a three-phase AC system, thus generating a magnetic field that rotates around the axis of the coil and has a strength that decreases with increasing distance from the coil (fig 3.3).

In a sensor coil, a voltage is induced proportionally to the time derivative of the magnetic field strength. Therefore the strains of the segments between the MFGC and the sensor measured by the coils can be calculated from the changes in the induced voltages. The angle $\angle$ (A'OC'; fig 3.2) equals the phase difference of the voltages induced in the sensor coils (fig 3.3).

The changes in the induced voltages of the sensor coils as well as their phase difference were registrated on a physiological recorder (Schwarzer), the former at 0.2, 0.4 or 0.8% per mm and the latter at 0.005 or 0.01 rad per mm. For normal cardiac contractions, the accuracy of the method is better than $\pm2\%$ for strain measurements and $\pm10\%$ for shear angle measurements (Arts and Reneman, 1980).

3.3.2.2 Assessment of epi and subendocardial fiber shortening

As discussed in section 2, torsion of the left ventricle is related to the transmural course of fiber shortening. The shear angle, as measured on the epicardial surface is directly related to this torsion. As will be shown in chapter 5, changes in shear angle after inducement of ischemia can vary between experiments, which might be due to differences in the initial orientation of the right angular configuration of the coils with respect to the base-apex direction. To be less dependent on this orientation, the principal strains $e_{min}$ and $e_{max}$ were calculated from $e_c$, $e_z$ and $\gamma$. The relation between these parameters is shown in fig 3.4. The ellipse determined by $e_c$, $e_z$ and $\gamma$, and represents the inscribed circle of the square after this square is deformed to the parallelogram. The short and long axis of this ellipse denote the direction of maximal ($e_{max}$) and minimal shortening ($e_{min}$), respectively. These parameters were calculated as follows: A first order approximation of the natural strain along a line forming an angle $\beta$ with the circumferential direction is

Fig 3.4
Circumferential strain, axial (or base-to-apex) strain and shear angle determine the deformation of a square into a parallelogram. From these three parameters, minimal strain, maximal strain and the angle of minimal strain ($\beta_{min}$) can be calculated, determining the deformation of the inscribed circle of the square (assumed at onset of ejection) into the ellipse (at the end of ejection). At the epicardium, orientation of maximal shortening is close to subepicardial fiber direction. The minimal shortening direction is close to the fiber direction in the inner layers.



Fig 3.5 Deformation of a circle (onset of ejection) into an ellipse (end of ejection) at the epicardium (left panel) and in the inner layers (right panel). Orientation of minimal and maximal shortening at the epicardium ($e_{min}$ and $e_{max}$, respectively) and in the inner layers ($e_{min,endo}$ and $e_{max,endo}$, respectively) is shown by drawn arrows. $\beta_{min}$ (outer layers) and $\beta_{min,endo}$ (inner layers) are the angles of minimal shortening. Shortening in the inner layers in the $\beta_{min}$ direction is called $e_{endo}$ (interrupted arrow). Orientation of the shortening directions is conform model calculations by Arts et al (1982a). Shortening as drawn in the figure is twice the calculated values.

$$e(\beta) = e_c \cdot \cos^2\beta + e_z \cdot \sin^2\beta + \gamma \cdot \cos\beta \cdot \sin\beta \qquad (1)$$

The maximal and minimal values of $e(\beta)$ are found by solving $de/d\beta = 0$, which results in

$$\tan (2\beta) = \gamma/(e_c - e_z)$$

Substitution of the thus found values of $\beta$ into equation (1) results in the maximal $(e_{max})$ and minimal values $(e_{min})$ of the natural strain, which are also the principal strains:

$$e_{min} = 0.5(e_c + e_z) - 0.5\sqrt{(e_c + e_z)^2 + \gamma^2}$$

$$e_{max} = 0.5(e_c + e_z) + 0.5\sqrt{(e_c + e_z)^2 + \gamma^2}$$

Experimentally (chapter 5) the orientation of $e_{max}$ was found to be close to the anatomically measured fiber orientation in the inner layers (Streeter et al, 1969). The fiber orientation in the inner layers is approximately perpendicular to the fiber orientation in the outer layers (Streeter et al, 1969). So the direction corresponding to $e_{min}$ at the epicardium is close to the fiber orientation in the inner layers. Thus, $e_{max}$ is close to shortening along the epicardial fiber direction and $e_{min}$ reflects shortening of the epicardial surface along the fiber direction in the layers.

## 3.3.3 Transmural differences in deformation

### 3.3.3.1 Principles

To measure directly endo and epicardial shortening perpendicular to the epicardial fiber direction, three needles were inserted through the myocardial wall so that they formed a rectangular triangle like the positioning of the magnetic coils used for measurement of epicardial surface deformation (section 3.3.2).

On each needle, two coils identical to those used for epicardial surface deformation (Arts and Reneman, 1980) were placed, 2 and 12 mm above the epicardium. So the deformation parameters were measured at two levels (fig 3.5). Deformation at the epicardium and in the inner layers was calculated by linear extrapolation along the needles from the measured deformation data.

Fig 3.6
Measurement of mutual mo-
vement and angulation of
needles pierced into the
myocardial wall during the
ejection phase is perfor-
med by determining circum-
ferential shortening,
base-to-apex shortening
and shear angle at two le-
vels above the epicardium
(straight lines). From
these measurements, the
three deformation parame-
ters can be calculated on
the epicardium and in the
inner layers by extrapola-
tion (dotted lines). Note
that the thus determined
epicardial parameters are
comparable with those mea-
sured directly on the epi-
cardium (fig 3.2).



Fig 3.7 Needle used for the measurement
of transmural differences in
deformation. Distance between
the coils is 10 mm.

Wall thickness was estimated from the relation between left ventricular weight and wall thickness as described by McHale and Greenfield (1973). For the calculation of deformation in the inner layers variations in wall thickness due to contraction or ischemia were neglected, because this technique was used as a first order approximation.

From the epicardial and subendocardial $e_c$, $e_z$ and $\gamma$ thus determined, $e_{min}$ and $e_{max}$ in these layers can be calculated as described in section 3.3.2.2.

Epicardial $e_{min}$ in these experiments was close to $e_{min}$, as calculated from data on epicardial deformation measurements (section 3.3.2.2). This $e_{min}$ forms an angle $\beta_{min}$ with the circumferential direction. Epicardial shortening along $\beta_{min}$ will probably be related with subendocardial shortening along $\beta_{min}$. Therefore, subendocardial shortening along the $e_{min}$ direction was calculated and defined as $e_{endo}$ (fig 3.6). Because the orientation of $e_{min}$ at the epicardium, and $e_{endo}$ is close to the fiber orientation in the inner layers (section 3.3.2.2), the relation between $e_{endo}$ and epicardial $e_{min}$ depicts the relation between subendocardial shortening along a direction close to the fiber orientation and shortening along the same direction on the epicardium.

### 3.3.3.2 Instrumentation

The stem of each needle consisted of a PVC cylinder (2mm outer diameter, 15 mm height). Underneath this stem a Tungsten needle (0.6 mm diameter, 25 mm length) was placed. To measure the mutual position of the needles, on each needle two coils were mounted 2 and 12 mm above the epicardium, respectively (fig 3.7). On the needle at the right angled corner, two magnetic field generating coils (MFGC) were mounted, and on the other two needles, two sensor coils were mounted. The same coils were used for the measurement of epicardial surface deformation (Arts and Reneman, 1980). It is known that insertion of needles of this size results in only little damage, as measured by histological and electrophysiological techniques (Franklin, 1974). To obtain fixation of the needle within the myocardium, small notches about 2 mm below the PVC cylinder, were made in each needle (fig 3.7). The electronic circuitry used in these experiments was the same as the one used in the measuring set-up in the previous section. So the amplitude of the changes of $e_c$, $e_z$ and $\gamma$ as measured during the cardiac cycle, could be registrated directly for only one level of coils at a time. Therefore, the signals from each level were alternatingly registrated with periods of 3-5 heart beats

using a switch. For each variable, the values of 2 or 3 beats were averaged.

## 3.4 Regional myocardial blood flow

### 3.4.1 Introduction

In 1967, Rudolph and Heymann introduced the microsphere method to measure regional blood flow with tracer microspheres of 50 $\mu$m in diameter. These spheres were injected into the circulation and were trapped in the smaller vessels. After the experiments, the organs were removed and their radioactivity counted. Repeated measurements could be made by using different nuclides and separating them by gamma-spectrometry. In several studies (Rudolph and Heymann, 1967; Domenech et al, 1969; Hoffbrand and Forsyth, 1969; Utley et al, 1972; Rudolph and Heymann, 1967; Buckberg et al, 1971; Yipintsoi et al, 1973) it was shown that the fraction of injected radioactivity retrieved in various organs was similar to the fraction of cardiac output reaching those organs during the transit of the spheres. If the flow to one organ and its microsphere content is known, the flow to any other organ or part of an organ, not in series with other microvascular beds, can be calculated.

Since both Heymann et al (1977) and Schamhardt (1980) reviewed the theoretical and practical aspects as well as the advantages and limitations of this technique, in the next sections the microsphere method is discussed only as far as relevant to the present study.

### 3.4.2 Choice of the type of microspheres

In this section, the choice of microspheres with respect to their size and specific activity will be discussed. Spheres with a diameter of 15 $\mu$m were used in the present study for the following reasons. When the transmural distribution of myocardial blood flow in the free wall of the left ventricle is investigated with microspheres, larger sized spheres systematically overestimate blood flow in the inner (endocardial) layers (Domenech et al, 1969; Utley et al, 1974; Yipintsoi et al, 1973). This has been ascribed to the more axial migration of larger spheres as compared with smaller ones (Segre, 1961; Phibbs and Dong, 1970). Because of this "streaming", larger spheres are preferentially deposited in areas with higher flow velocities (Fung, 1973). Since the outer layers receive blood during the whole cardiac cycle and the inner layers only during diastole, and mean blood flow to both areas is almost equal, it is evident that flow velocities are higher in branches

supplying the inner layers at equal vessel diameters (Estes et al, 1966). Smaller spheres which migrate more peripherally in the transmural arteries (originating from the larger epicardial coronary arteries) might be trapped preferentially in more proximal (epicardial) branches. With diffusable tracers, endo/epicardial blood flow ratios have been found to be above unity in the isolated dog heart preparation (Yipintsoi et al, 1973) as well as in the intact dog heart (Prokop et al, 1974). Microspheres with a diameter of 9 $\mu$m give endo/epi ratios which are well comparable with those obtained with diffusable tracers (Heymann et al, 1977: fig 3). According to this figure, 15 $\mu$ beads give a slight overestimation of this ratio. Fan et al, (1979) showed that in the outer layers blood flow values obtained with 9 and 15 $\mu$m spheres are comparable, but values in the inner layers are about 10% higher for the 15 $\mu$m spheres as compared with 9 $\mu$m spheres.

On the other hand, a relatively high arterio-venous shunting was found when using 9 $\mu$m spheres varying between 1 and 6% under normal conditions (Utley et al, 1974; Crystal et al, 1979; Downey et al, 1979; Fan et al, 1979; Tripp et al, 1977) and increasing to 8% during elevation of arterial $p_{CO2}$ (Fan et al, 1979) or increased perfusion pressures, up to 26.7 kPa (Crystal et al, 1977). In the myocardium, the untrapped fraction of 15 $\mu$m microspheres was always less than 2% (Fortuin et al, 1971; Utley et al, 1972; Fan et al, 1979), even at elevated arterial $p_{CO2}$ (Fan et al, 1979).

In this study, microspheres with a diameter of 15 $\mu$m were used because they seem to be the best compromise between the validity of transmural blood flow measurement and the entrapment of microspheres in the small vessels. Moreover, microspheres of 15 $\mu$m are less expensive than the smaller sized ones.

The specific activity of the microspheres used in this study is lower than that used in other investigations. The lower specificity was selected because of the lower radiation load for the investigators. At a specific activity of 3 mCi.g$^{-1}$ (weight of spheres), the radioactivity in the spheres is sufficiently high to acquire an accurate gamma counting. In fact, at specific activities down to 0.5 mCi.g$^{-1}$, the number of microspheres per sample and not the gamma counting is the main determinant of the accuracy of the method. An additional advantage is that less radioactivity has to be injected to obtain a certain number of spheres per sample. The price per number of microspheres is more favorable at the lower specific activities as well.

3.4.3 Checks before experimental use

The microspheres were purchased from either the 3M Company (Minnesota, USA) or New England Nuclear (Boston, USA). Microspheres were delivered in injection vials, suspended in 10% Dextran with 0.05% Tween 80 as surfactant to reduce aggregation of the spheres. Before use in experiments, each batch was checked on: (a) purity of the nuclide, (b) specific activity of the microspheres, (c) integrity of the spheres, (d) leakage of the nuclides from the spheres and (e) diameter distribution of the beads, according to Heymann et al (1977).

ad (a) Purity of the nuclide is required for accurate gamma spectrometry. The purity was checked by analysis of the spectrum of a small sample of each batch on the monitor of the Multi-Channel Analyzer. In each case, the right nuclide was delivered and no contamination with other nuclides was found.

ad (b) Of each batch of microspheres the specific activity was measured to check whether the materials delivered were according to the manufacturer's specifications. Furthermore, for accurate determination of the number of microspheres in each blood or tissue sample the specific activity of the beads has to be known precisely. For this determination, a droplet of a diluted suspension of microspheres was spread on a piece of graph paper. The number of microspheres (usually 1000-2000) on this piece of paper was counted microscopically at an enlargement of 100x. After this procedure, the radioactivity of this piece of paper was counted in a gamma counter. Subsequently, it served as an isotope reference sample during counting of each series of samples from an experiment (section 3.4.4).

ad (c) Damaged microspheres may have other flow behavior, may fail to be entrapped and may cause leakage of the nuclide from the spheres. These factors negatively influence the accuracy of the method. Therefore, microsphere integrity was checked microscopically in a small sample at an enlargement of 100x. A few hundred beads were checked per sample. In each case, less than 1% damaged spheres were observed.

ad (d) Substantial leakage of nuclides from the spheres will invalidate the method because in this case not only radioactivity is injected which is trapped by the microcirculation, but also soluble radioactivity which is recirculating.

This leakage was checked as follows. A sample of microspheres was

taken from batch, sedimentated and once washed (to mimic the treatment of microspheres to be injected in the animal, section 3.4.4). After a few ml 10% Dextran with 0.05% Tween 80 was added and the tube was thoroughly shaken on a Vortex mixer during 1 min and sonificated in an ultrasound bath during 5 min, this tube was counted. Thereafter, the suspension was shaken again on the Vortex and subsequently filtered through a 0.22 $\mu$m millipore filter (Cathivex). The supernatant was counted and from its activity divided by the activity of the whole microsphere sample the percentage leakage was calculated. This acute leakage was maximally 0.15%. The mean value of acute leakage was 0.03% with a s.d. of 0.05%, for 3 M and NEN spheres combined. These values were lower than the specifications of the manufacturers (0.16% $\pm$ 0.12%, x $\pm$ s.d.).

The leakage at $37^O$ C over longer periods of time was determined in four batches of microspheres. After vigorous shaking, samples of these batches were stored in a $37^O$ waterbath for 1 to 2 weeks. Intermittently, the suspensions were shaken about five times. After this period, leakage was determined in the same way as in the acute leakage experiments. Values of longterm leakage were found to be about twice as high as those for the acute leakage which is in agreement with the manufacturer's specifications, mentioning a leakage of less than 1%. These results show that microspheres will loose only a negligible portion of their radioactivity in an animal over a period of 2 hours. This is supported by the observation that blood, withdrawn one hour after the last microsphere injection, contained no substantial radio-activity.

ad (e) Diameter distribution of the microspheres. The importance of a narrow diameter distribution of each batch of microspheres has been emphasized by Reneman et al (1975). Because beads of different size have different rheological properties (section 3.4.2), a large variation in microspheres size will also introduce larger coefficients of variation.

For this reason and also to check whether the right size of spheres was delivered, in each batch the diameter distribution was determined in a sample of 50 microspheres. Initially, this measurement was performed with an ocular micrometer in the light microscope using a magnification of 400-600x. The ocular micrometer was calibrated with

an objective micrometer. However, the accuracy of this method was 0.5 $\mu$m due to physical limitations of the light microscope. Therefore, later in this study the diameter of the spheres was measured with an imagesplitting eyepiece (Vickers A.E.I.) mounted on a light microscope. This reduced variations in the diameter measurement to almost 0.1 $\mu$m. The observed diameters and their distributions were comparable with the data provided by the manufacturers.

### 3.4.4 Experimental use of microspheres

The number of spheres in each batch was calculated using data concerning total activity (mCi) and specific activity (mCi.g$^{-1}$) as well as the number of spheres per gram as provided by the manufacturer. From each batch a stock suspension in fresh 10% Dextran with 0.05% Tween 80 was made containing $5 \times 10^6$ microspheres per ml. Prior to each experiment, the wanted number of microspheres was taken from this stock solution and diluted twice in 10% Dextran in a 5 ml syringe.

Before injection, the syringe, which was closed by a stopcock, was thoroughly shaken on a Vortex mixer to suspend all microspheres. Then the syringe was put into a beaker in an ultrasonic bath. The beads were sonificated for 5-10 min to break up clumps of spheres (Reneman and Verheyen, 1977). Shortly before injection, the syringe was taken out of the bath and again shaken on the Vortex mixer for about 1 min. Within 1 min after stopping this procedure, the microspheres were injected into the left atrium via a silastic catheter (inner diameter 2 mm, length ca. 25 cm). The syringe was washed once with about 3 ml physiological saline and subsequently the catheter was flushed with another 7 ml of physiological saline to remove the microspheres.

An arterial reference sample was taken from the brachial artery at a rate of about 20 ml.min$^{-1}$, using a Harvard suction pump. Withdrawal of blood started 5 s before the injection of the microspheres and was continued during at least 1 min. The arterial blood was put into test tubes and the sample syringe was flushed once with a few ml of water. After killing the animal by an overdose of barbiturate, the heart was excised, rinsed and stored in formaldehyde 5%. The free wall of the left ventricle was cut into 5 slices (parallel to the base of the heart). Each slice was cut into 5 pieces of approximately 3 gram. Each piece was divided into a subendocardial, middle, and subepicardial layer (see fig 3.8). The tissue samples were put into PVC 10 ml test tubes. These tubes were weighed empty and with the sample. The

Fig 3.8 Schematic drawing of the method of dividing the left ventricu-
lar free wall. The dotted area indicates the position of the
papillary muscles. 1-5 are called slices, A-E are called sec-
tions.



Fig 3.9 Parts from the output of the MICRO-program for the calculation
of regional myocardial blood flow, showing the values of myo-
cardial blood flow (ml.min$^{-1}$.g$^{-1}$) and the endo/epicardial
blood flow ratio according to the location of the samples in
the left ventricular free wall (see fig 3.8).

weighing results were typed in on a data-entry system with a portable memory (3.84 kbytes).

In the Packard Multi-Channel Analyzer the following samples were counted according to the MIC II program input (Schosser et al, 1979): background samples, isotope reference samples, substract samples (meant for empty syringes), arterial and venous reference samples and tissue samples.

To be able to distinguish between the radioactivity of different nuclides within one sample as much counting windows were programmed as isotopes were present. Each window was located around the photopeak(s) of an isotope, taking into account that the overlap of other nuclides in that window was as small as possible. The spectrum of each isotope could be visualized on a display, facilitating the positioning of the windows.

### 3.4.5 Calculation of regional blood flow

Data obtained from the gamma counter were sample number in the gamma counter, counting time and the gross counts in each window. These data were printed on paper and on papertape. The papertape data were fed into the MINC II computer, using a Facit papertape reader, and stored on file on a floppy disc.

The weighing data in the portable memory were read into the computer by a data entry (manufactured at the University of Limburg) in octal code and subsequently converted into alpha numeric figures. By a screening program, these data were checked on errors in the input and the data were ranked in negative numbers (for the empty tubes) and positive numbers (for tubes with samples) for direct use in the MICRO program.

The MICRO program is based on the MIC II program of Schosser et al (1979). Shortly, the analysis of the energy spectra is performed according to the matrix method. Isotope reference samples (containing each a certain amount of one pure isotope) are measured to obtain the overlap matrix. The unknown amounts of the nuclides in the samples are calculated by solving a set of linear equations. The original MIC II program has been changed for convenience on a few points:

a. Calculation of the ratio count per min (cpm)per microsphere. This ratio was originally determined by dividing the activity of the whole injectate by the estimated number of injected microspheres. Direct gamma counting of the injectate however is not feasible when a large experimental animal is

used, because a high dose of radioactivity is required. Therefore, from each nuclide 1000-2000 microspheres were counted microscopically on a piece of graph paper and after counting put into a counting tube. Manual input into the MICRO program of this number of microspheres, together with the papertape input of the counting data results in the cpm/microsphere.

b. Determination of the injected numstract samples) to correct for microspheres remaining in the syringes is not necessary. Thus in the present study, substract samples were replaced by empty tubes. The injected number of microspheres was estimated from the volume of the stock suspension used for injection.

c. Because the left ventricular free wall is divided systematically into 75 pieces (section 3.4.4) it was possible to make a "map" of MBF in each experiment. To provide this "map", the MICRO program was extended to obtain geographic information about the distribution of MBF in the free wall of the left ventricle (see fig 3.9).

The output of the computer was stored on file and subsequently printed. Beside the data provided in the original version of the MICRO-program (Schosser et al, 1980). Also the MBF values and the endo/epicardial blood flow ratios of the 25 transmural pieces of all 75 samples are given (fig 3.9).

Because prior to dissection of the heart the tissue samples in which biopsies had been taken for the assessment of ATP, creatine phosphate and glycogen or fatty acid content, had been marked, the MBF values of these samples could be related to these biochemical variables. Total MBF in the ischemic and non-ischemic area was calculated as well. The ischemic area was considered to be the zone perfused by the left anterior interventricular coronary artery which was identified macroscopically. For the determination of total flow in this ischemic zone, only samples in which during stenosis flow in the subendocardial part of the transmural section was reduced by at least 40% as compared with the pre-stenotic period, were taken into account. The non-ischemic area was considered to be the zone of tissue along the left circumflex coronary artery and along the marginal branch of this artery (fig 3.8). The OVERAL computer program grouped tissue samples per layer so that beside total flow, MBF in the three layers of the non-ischemic and ischemic zones could be calculated as well as the mean endo/epicardial blood flow ratio.

3.4.6 Accuracy of the method

Variations in the assessment of regional blood flow by radioactive micro-
spheres originate mainly from two factors: properties of the microspheres and
measurement of the presence of these spheres. Variations in the latter one
originate from errors in gamma counting of tissue and blood samples, in the
weighing of the tissue samples and in the matrix calculations.

The weight of the tissue samples is determined with an accuracy of 1 mg
introducing a variation of about 0.1% in the tissue samples (mean weight
about 1 g).

For a proper determination of the radioactivity of a sample, the back-
ground activity has to be known as well. This background activity shows
relatively large variations within the counting times used (10 min). For
$Ce^{141}$, $Sn^{113}$, $Ru^{103}$, and $Nb^{95}$, the variation in the background activity was
12.4, 5.5, 5,8 and 6.6%, respectively. However, in tissue samples containing
radioactivity of at least 10 times the background, the assay variation is
less than 2%. Because the background in each window ranged from 10-40 cpm,
only in samples with a small amount of spheres (samples from severely ische-
mic tissue, for example) this variation has to be taken into account.

Errors due to the matrix calculations providing corrections for Compton
scatter were evaluated in a simulation experiment. The input data for the
isotope reference samples were chosen to mimic the nuclides $I^{125}$, $Sr^{85}$, and
$Nb^{95}$. The relative activity of the different nuclides in the simulated tissue
samples were varied to simulate flow differences up to a factor of 10 at the
different injection times. This error was 1.0% for $I^{125}$ and less then 0.01%
for the other three isotopes. For experiments where 8 isotopes were used,
Schosser et al (1979) reported errors of less than 0.23%.

In contrast to the above-mentioned factors, errors caused by properties of
the microspheres mainly determine the variations in the results obtained with
the microsphere method: inhomogeneous distribution of the spheres over the
cross-sectional area of the blood vessel, incomplete mixing of spheres in the
blood and statistical variation in microsphere distribution.

Inhomogeneous distribution of microspheres over the cross-sectional area
of the vessel is responsible for differences in transmural distribution
across the left ventricular wall between different sizes of microspheres
(section 3.4.2).

To check adequate mixing of the microspheres in the blood, 6 experiments
were performed. In 3 experiments, 4 isotopes were simultaneously injected. In

each of the 3 experiments, activity was 4.7% ± 5.0%; 1.8 ± 1.6% and 0.2 ± 1.4% higher in the femoral than in the brachial artery sample (mean and s.d. of the 4 isotopes). In 3 other experiments, 4 isotopes were injected subsequently, at an interval of 5 min during a stable hemodynamic period. In these experiments activity was 2.1% ± 3.3% lower; 2.6 ± 2.5% and 1.9 ± 0.6% higher in the femoral than in the brachial artery sample. These results indicate that microspheres are not preferentially distributed to either the brachial or the femoral artery, suggesting a homogeneous distribution of microspheres in the blood, which is in agreement with results obtained by other investigators (Hoffbrand and Forsyth, 1969; Buckberg et al, 1971). These experiments also show that between different microsphere injections a coefficient of variation of 3.5% exists.

Because microspheres are distinct particles, variations in the concentration of spheres occur as a result of statistical variation even with homogeneous blood distribution. This variability approximates the Poisson distribution (Buckberg et al, 1971). This implies that the variation in the microsphere method is dependent on the number of spheres in each sample (Buckberg et al, 1971; Reneman et al, 1975; Flameng et al, 1977). To investigate whether in our experimental set-up this variation is comparable to those found by these authors, in 2 experiments 4 isotopes were injected simultaneously into the left atrium. Before this injection, the LAICA was stenosed to create inhomogeneous flow distribution within the left ventricle resulting in large differences in microsphere content of the tissue samples. The results of these experiments are shown in table 3.1. The differences in calculated myocardial blood flow (MBF) values between 2 isotopes in each sample are expressed as the relative error (E) or as coefficient of variation. The relative error was calculated according to the following equation (De Jonge, 1964):

$$E = 1.96 \cdot \frac{\sqrt{\Sigma \ di^2/2k}}{\bar{x}} \cdot 100\%$$

where $d_i$ = the difference in calculated MBF values between 2 isotopes in the various samples, k = the number of samples and $\bar{x}$ = the mean MBF calculated from the 2 isotopes.

Table 3.1 Coefficient of variation and relative error (section 3.4.6) of myocardial blood flow values obtained with any two isotopes after simultaneous injection of four isotopes during coronary artery stenosis.

| Number of micro-spheres per iso-tope per sample | Coefficient of variation (%) | Relative error (%) | Number of comparisons |
|---|---|---|---|
| > 2000 | 3.2 | 7.1 | 72 |
| 1000-2000 | 3.6 | 8.2 | 81 |
| 700-2000 | 5.0 | 7.0 | 30 |
| 400-700 | 5.6 | 9.9 | 24 |
| 200-400 | 6.4 | 15.5 | 56 |
| 100-200 | 9.0 | 16.0 | 22 |
| <100 | 30.0 | 70.3 | 78 |

Table 3.2 Coefficient of variation of myocardial blood flow values obtained with any two isotopes, separately injected during a stabile hemodynamic situation after coronary artery stenosis.

| Number of microspheres per isotope per sample | Coefficient of variation (%) | Number of comparisons |
|---|---|---|
| >2000 | 12.8 | 14 |
| 1000-2000 | 10.7 | 31 |
| 700-1000 | 11.8 | 11 |
| 400-700 | 13.8 | 15 |
| 200-400 | 15.0 | 16 |
| 100-200 | 20.7 | 8 |
| <100 | 40.4 | 9 |

The coefficient of variation was calculated according to the equation:

$$C.V. = s.d./\ \overline{x}$$

where s.d. = standard deviation and $\overline{x}$ = mean calculated MBF.

Tissue samples were classified in groups containing <100, 100-200, 200-400, 400-700, 700-1000, 1000-2000 and > 2000 microspheres per sample. No significant differences were observed between the MBF values of the 4 isotopes. Therefore, the analysis of the results from the comparison between each pair of isotopes was combined (see table 3.1).

These results show that in samples containing at least 400 microspheres, variation of the MBF determination is less than 10%. This value is comparable to the values found by Buckberg et al, 1971 (in arterial reference samples), Flameng et al, 1977 (in tissue samples from an isolated heart preparation), and Reneman et al, 1975 (in a set-up identical to the one used in this study). In comparison with the latter investigators, the relative error in our experiments tends even to be less. This might be due to a more narrow diameter distribution of the spheres used in this study.

It has to be emphasized that the variations found in these 2 experiments are the sum of all experimental variations mentioned above, except for the variations in arterial reference sampling. However, the other variations are not influenced or only to a limited extent to the number of microspheres in one sample. Thus, the variations shown in table 3.1 are the intra-assay variations for a certain number of microspheres.

The inter-assay variation theoretically will be equal to the intra-assay variation plus the arterial reference sample variation. This variation could be determined by injecting the isotopes not simultaneously but separately at short time intervals. However, in this case, no discrimination can be made between the inter-assay variation and real changes in flow even in an apparent stable hemodynamic situation. Nevertheless, we investigated these variations in 2 experiments, while the LAICA was stenosed. Variation coefficients in samples with different microsphere contents are shown in table 3.3. Again, the relation between the number of spheres per sample and the variation coefficient is clear. However, comparison with the data from table 3.1 show that for any number of microspheres the variation coefficient is 7-10% higher in case of separate injections. This difference will be caused by variations

if regional blood flow values as obtained from 3M
N (Ru[103] and Sn[113]) microspheres. Shown are the
organs in the systemic circulation (■) and from
of the 4 animals.

Table 3.3 Relative errors (section 3.4.6) in MBF values from organs in the systemic circulation, obtained after simultaneous injection of three isotopes. n depicts the number of comparisons.

| Number of microspheres per sample | Nb - Sn (3M) (NEN) | n | Nb - Ru (3M) (NEN) | n | Sn - Ru (3M) (NEN) | n |
|---|---|---|---|---|---|---|
| >2000 | 3.6 | 16 | 6.0 | 2 | 4.5 | 2 |
| 1000-2000 | 4.6 | 11 | 4.6 | 9 | 5.8 | 9 |
| 400-1000 | 12.0 | 8 | 3.7 | 11 | 2.3 | 11 |
| 100-400 | 21.8 | 12 | 7.9 | 13 | 2.9 | 13 |
| <100 | 29.2 | 11 | 14.4 | 14 | 15.5 | 14 |

in arterial reference sampling as well as in the hemodynamic situation. In other experiments, the variation in arterial reference sampling have been shown to be 3.5% so even in an apparent stabile situation, variations in MBF of 3.5-6.5% seem to be common and should be emphasized in interpreting results obtained with the microsphere method.

3.4.7 Comparison between 3M and NEN microspheres

Until recently, the only brand of non-biodegradable spheres available and extensively investigated on their validity for the measurement of regional blood flow were those manufactured by the 3M Company. Since a few years, NEN also delivers radioactive microspheres which are claimed to be at least as reliable as the 3M spheres. Knowledge about the exact composition of either brand of spheres is absent. The 3M spheres are made of "an inert plastic material" which is carbonized; the NEN spheres consist of "styrene-divinyl benzen copolymer coated with a polymeric resin".

However, in using any type of microspheres to measure regional blood flow, a number of assumptions are implicit. Since 3M microspheres have been extensively investigated and validated (section 3.4.1), a comparison between the results obtained with 3M and NEN spheres was made.

The comparison of both types of microspheres was performed in 4 rabbits (mean weight 4 kg). The animals were anesthetized with 0.4 ml.kg$^{-1}$ Rompun and 0.2 ml.kg$^{-1}$ Vetalar and ventilated after endotracheal intubation with a pediatric positive pressure respirator. The thorax was opened through the left 5th intercostal space and the pericardium was opened. For injection of the microspheres a small silastic catheter was inserted into the left atrium. A polyethylene catheter was inserted into a brachial artery to obtain an arterial reference sample. With a syringe, a mixture of 15 $\mu$ microspheres labeled with Nb$^{95}$ (3M), Ru$^{103}$ (NEN) and Sn$^{113}$ (NEN) was injected into the left atrium. The reference sample was taken at a rate of 6.0 ml.min$^{-1}$, using a Harvard suction pump. Withdrawal of blood started 5 s before injection of the spheres and was continued during at least 1 min.

After the experiment, tissue samples of different size were taken from the heart, kidneys, liver, spleen, skeletal muscles and lungs. From these samples, regional blood flow was determined as described in section 3.4.5.

The coefficients of variation in blood flow values in the heart alone as well as these values combined with blood flow values in the liver, kidneys and skeletal muscles are shown in table 3.3. The variations in blood flow

values in these organs were combined to obtain in each class of number of spheres per sample a reasonable number of observations. Moreover, no consistent differences were observed between the variations in the different organs. The obtained values of the relative error are comparable to those found after simultaneous injection of 3M microspheres with 3 different isotopes (table 3.1).

Linear regression analysis showed an excellent relation between the obtained MBF values of 3M and NEN spheres (see fig 3.10). Also entrapment of either type of microspheres in the lungs was almost identical, suggesting that no differences in shunting through the systemic circulation were present.

According to these results, it was concluded that measurement of regional blood flow with NEN microspheres has the same validity as 3M microspheres. This is in agreement with the results of Hales et al (1979) in sheep.

## 3.5 Determination of chemical substances in blood

### 3.5.1 Introduction

The determination of several substances in the blood was performed for several reasons. Firstly, assessment of myocardial substrate utilization requires the measurement of the uptake of oxygen, glucose, lactate and fatty acids by the myocardium (section 2.3). As mentioned in section 3.7, the uptake of these substances was estimated from the product of myocardial regional blood flow and the respective arteriolocal venous (AV) differences of these substances. Moreover AV differences of NEFA were determined to investigate to which extent the accumulation of NEFA during ischemia, as observed in a previous study is caused by uptake of these NEFA(Van der Vusse et al, 1982). Secondly, the release of lactate, inorganic phosphate, potassium, $CO_2$ and $H^+$ was determined because these substances are known to be good biochemical markers of myocardial ischemia.

At last, the determination of blood gases, pH and oxygen content of arterial blood was used to check the respiratory and metabolic condition.

### 3.5.2 Blood gases and blood pH

The blood pH was determined potentiometrically using a pH glass electrode and a calomel reference electrode. The $p_{CO2}$ was measured using a $p_{CO2}$ electrode, in fact a pH electrode the surface of which can only be activated by

Table 3.4 Precision of the determination of several substances in the blood. For calculation of the coefficient of variation, see section 3.4.6.

| Variable | Intra-assay coefficient of variation (%) |
|---|---|
| $P_{CO2}$ | 2.3 |
| $P_{O2}$ | 2.3 |
| Hb | 2.8 |
| $O_2$ saturation | 0.5 |
| Glucose | 1.1 |
| Lactate | 2.2 |
| Inorganic phophate | 1.4 |
| Potassium | 0.6 |
| pH | 0.007 units |

Table 3.5 Precision of the gaschromatographic method for the estimation of NEFA in dog serum (8 replicate assays).

| Sample number | Mean amount of NEFA estimated ($\mu$mol.1$^{-1}$) | SD ($\mu$mol.1$^{-1}$) | Intra-assay coefficient of variation (%) |
|---|---|---|---|
| I | 278 | 4 | 1.4 |
| II | 179 | 4 | 2.2 |
| III | 123 | 5 | 3.9 |
| IV | 103 | 3 | 2.6 |
| Mean | | | 2.5 |

Table 3.6 Precision of the GLC method for estimation of individual fatty acids of the NEFA class in dog serum (8 repl. assays)

| | Intra-assay coefficient of variation (%) | | | | |
|---|---|---|---|---|---|
| Sample number | I | II | III | IV | mean |
| NEFA: | | | | | |
| Myristic acid | 5.3 | 10.3 | 21.9 | 10.8 | 12.0 |
| Palmitic acid | 4.5 | 5.2 | 2.4 | 2.1 | 3.6 |
| Palmitoleic acid | 1.9 | 3.6 | 4.4 | 7.3 | 4.3 |
| Stearic acid | 2.4 | 4.6 | 4.9 | 3.5 | 3.8 |
| Oleic acid | 1.4 | 3.3 | 2.9 | 1.5 | 2.2 |
| Linoleic acid | 3.6 | 3.3 | 2.8 | 2.7 | 3.1 |
| Arachidonic acid | 28.6 | 7.8 | 14.7 | 9.3 | 15.1 |

uncharged molecules due to a thin Teflon outer membrane. Determination of $p_{O2}$ was performed with an amperometrical method. Intra-assay coefficients of variation of these three methods are shown in table 3.4. Determination of the blood gases and blood pH was performed at 37 C using a blood gas analyzer (IL 413 or Radiometer ABL3).

### 3.5.3 Hemoglobin, oxygen saturation and oxygen content

These measurements were performed using a Radiometer OSM 2 hemoximeter. The method is based on spectrophotometric analysis at 505 and 600 nm at $37^{O}C$. The molar absorbances of Hb and $HbO_2$ are identical at 505 nm but widely dif ferent at 600 nm. Intra-assay coefficient of variation of the $O_2$ saturation determination was 0.5% and of the hemoglobin determination 2.8% (table 3.4).

The oxygen content of the blood was definedasthe product of hemglobin concentration oxygen saturation. Since the amount of 02 in the plasma is smaller compared to the hemoglobin bound oxygen.

### 3.5.4 Glucose, lactate, inorganic phosphate and potassium

The concentration of these substances in the serum of the blood samples was determined by spectrofotonetry on a Technicon AAII Auto-analyzer.

Glucose was determined with hexohinase and glucose-6-phosphate dehydroge- nase as described by Bergmeyer et al, (1974).

Intra-assay coefficient of variation of the blood glucose determination was 1.1%. Lactate was determined with lactate dehydrogenase as described by Gutmann and Wahlefeld, (1974), modified for assessment on the Auto-analyzer. Intra-assay coefficient of variation of this method was 2.2%.

Serum inorganic phosphate concentrations were determined with ammonium molybdate according to Fiske and Subbarow (1926). These complexes were spec- trophotometrically assessed. For this method, the intra-assay coefficient of variation was 1.4% (table 3.4).

Concentrations of potassium in the serum of blood samples were determined with a Technicon Auto-analyzer Flame Photometer IV coupled to the above-men- tioned Auto-analyzer. The method is based on a procedure described by Berry et al (1946). Intra-assay coefficient of variation of this determination was 0.6% (table 3.4).

Materials for the glucose, inorganic phosphate and potassium determination were purchased from Technicon, Tarrytown. For the lactate determination LDH was purchased from Boehringer, Mannheim, and lactate from Sigma, St. Louis,

and the other chemicals from Merck, Darmstadt.

### 3.5.5 Non-esterified fatty acids

The concentration of non-esterified fatty acids (NEFA) in blood can be determined using a colorimetric, titrimetric, radiometric, enzymatic or gaschromatographic method. Initially, this concentration was determined using a colorimetric method (data presented in chapter 8). However, in the course of this study, we preferred the gaschromatographic method (other data),giving also data concerning the individual NEFA. The colorimetric assay was performed with the  standard Boehringer test kit (Boehringer, Germany). NEFA form with copper nitrate salts which are soluble in chloroform. With diethylthiocarbaminate, yellow complexes are formed, the color intensity of which can be measured spectrophotometrically at 420nm. The determination was started immediately after the samples were thawed. For each determination, 0.1 ml of serum was used. The intra-assay coefficient of variation was 4%.

For gaschromatographic determination of the serum concentration of NEFA, $100 \mu l$ serum was extracted with 2.0 ml methanol/chloroform (1:2, v/v) at room temperature. Serum extracts were subsequently treated in a similar fashion as the extracts of myocardial biopsies (section 3.6.4). In table 3.5 and 3.6, respectively, the standard deviation and intra-assay coefficient of variation of the total NEFA determination and of the individual NEFA is shown for 4 samples, each value being calculated from 8 replicate assays. The high coefficient for myristic acid and arachidonic acid is caused by their very low concentrations in the blood. Manufacturers of the materials used for this determination are mentioned in 3.6.4.

### 3.5.6 Triacylglycerol

After thinlayer chromatography of the serum, the triacylglycerol spot was scratched off, the fatty acid moieties were transmethylated and gaschromatographically determined (for conditions during this determination, see section 3.6.4). Intra-assay coefficient of variation of this method varies from 1.4 to 2.8% (mean 2.2%), as calculated from 8 replicate assays of 4 samples (table 3.7).

Manufacturers of the materials used for this determination are mentioned in 3.6.4.

Table 3.7 Precision of the gaschromatographic method for the estima-
tion of fatty acids of triacylglycerol in dog serum (8
replicate assays).

| Sample number | Mean amount of FA estimated ($\mu$mol.$1^{-1}$) | SD ($\mu$mol.$1^{-1}$) | Intra-assay coeff. of var. (%) |
|---|---|---|---|
| I | 750 | 20 | 2.7 |
| II | 733 | 12 | 1.6 |
| III | 721 | 6 | 1.4 |
| IV | 670 | 19 | 2.8 |

Table 3.8 Precision of the determination of several substances in
myocardial tissue

| Variable | Intra-assay coefficient of variation (%) |
|---|---|
| ATP | 4.7 |
| CP | 3.3 |
| Glycogen | 3.8 |
| NEFA | 2.3 |
| Triacylglycerol | 6.5 |
| Phospholipids | <7.2[*] |

[*] Difference between two samples in the same heart (see text)

Table 3.9 Precision of the gaschromatographic method for the estima-
tion of the individual fatty acids of the NEFA class in
normoxic dog myocardial tissue

| | Intra-assay coefficient of variation (%) | | | |
|---|---|---|---|---|
| Sample number | I | II | III | mean |
| Number of measurements | 4 | 2 | 2 | |
| NEFA: | | | | |
| Myristic acid | 36.1 | 21.1 | 3.1 | 20.1 |
| Palmitic acid | 7.6 | 1.2 | 9.6 | 6.1 |
| Palmitoleic acid | 18.9 | 15.7 | 13.9 | 16.2 |
| Stearic acid | 3.3 | 11.1 | 3.7 | 6.0 |
| oleic acid | 4.7 | 4.5 | 7.3 | 5.5 |
| linoleic acid | 9.5 | 4.9 | 0.5 | 4.8 |
| arachidonic acid | 24.1 | 30.1 | - | 27.1 |

## 3.6 Determination of chemical substances in myocardial tissue

### 3.6.1 Introduction

Tissue samples have been taken for two purposes. The first was to obtain information about energy stores present in the myocardium during ischemia. For this purpose transmural biopsies (about 100 mg) were taken. These samples were divided into samples from the outer, middle and inner layers, in which ATP and creatine phosphate concents were determined. To obtain information about the carbohydrate store, glycogen concent was determined in the same biopsies.

The second purpose was to investigate the time course of the ischemia induced changes in tissue concents of fatty acids in the non-esterified fatty acid, triacylglycerol and phosphoglyceride classes. For this purpose, separate biopsies were taken, because these determinations require relatively large tissue samples (see below). These biopsies were divided into three parts as well.

### 3.6.2 Handling of the tissue samples

Before chemical analysis the tissue samples for ATP, creatine phosphate and glycogen determination were freeze dried within the drills in a Virtis freeze dry apparatus. Care was taken that during this procedure the biopsies stayed at a temperature not higher then $-30^{\circ}$C until they were dry. Thereafter the biopsies were pushed out of the drills and divided into samples from the outer, middle and inner layers. To reduce interference with the chemical analysis, adherent dried blood was removed as much as possible under a binocular microscope using a small pincet and a needle. Subsequently, these samples were stored again at $-80^{\circ}$C or directly used for chemical analysis.

### 3.6.3 ATP, creatine phosphate and glycogen

The dried tissue was crushed with a glass rod in a 0.2 ml water-acetone (1:1, v/v) mixture, containing 2 mM EDTA and 0.4 M perchloric acid at a temperature of $-15^{\circ}$ C. The whole mixture was subsequently neutralized with 0.1 ml ice-cold TRIS-KOH and centrifuged for 10 min at $4^{\circ}$ C with a relative centrifugal force of 1100 g. The clear supernatant was carefully removed and aliquots were used for the determination of ATP and creatine phosphate. The residue containing fragments of tissue and insoluble salts was used for the determination of glycogen (vide infra).

ATP and creatine phosphate were analyzed with a fluorometric technique (Lamprecht and Trautschold, 1974; Lamprecht et al, 1974), modified by Drake et al (1980b). The reaction mixture per liter was: TRIS buffer (55 mmol, pH=8.1), $NADP^+$ (1 mmol), glucose (1 mmol), $MgCl_2$ (1 mmol) and glucose 6-phosphate dehydrogenase (160 U). After addition of 10 $\mu$l tissue extract to 1.0 ml reaction mixture, the reaction was started with hexokinase (690 mU). The amount of ATP was calculated by comparison of the change of fluorescence in the test cuvet with the standard cuvet (100% fluorescence has been standardized with 5 nmol NADPH in 1.0 ml buffer). From a subsequent addition of ADP (final concentration 0.05 mol) and creatine kinase (980 mU) the amount of creatine phosphate was determined in the same cuvet.

The afore-mentioned residue (see ATP and creatine phosphate determination) was kept at $37^o$ C for 3 hours after addition of 1.0 ml 1.0 M NaOH. Subsequently, 1.0 ml 95% ethanol was added and the whole mixture was heated for 10 min at $85^o$ C. After cooling to $4^o$ C, the mixture was stored at this temperature for 24 hours. The mixture was centrifuged at $4^o$ C with 1100 g for 20 min. The clear supernatant was carefully removed with a pipette, and the residue was washed twice with 0.5 ml 60% ethanol. After centrifugation at $4^o$ C with 1100 g for 30 min, the residue was hydrolyzed at $85^o$ C for 3 hours after addition of 1.0 ml 1.0 M HCl to the residue. The mixture was subsequently neutralized with 0.5 ml TRIS-KOH and aliquots were used for the fluorometric assay of the glucose moieties.

Instead of glucose, ATP (1 mM) has been added to the afore-mentioned reaction mixture. After addition of 10 $\mu$l tissue extract, the reaction was started with hexokinase (690 mU).

The determinations were performed with a Zeiss ZFM4 Fluorometer, at a temperature of $25^o$ C. The intra-assay coefficient of variation of the determination of ATP, creatine phosphate and glycogen are shown in table 3.8.

Materials
Enzymes hexokinase (140 U/mg), creatine kinase (25 U/mg) and glucose 6-phosphate dehydrogenase (350 U/mg) were purchased from Boehringer Mannheim, β-nicotinamide-adenine dinucleotide phosphate (disodium salt) and adenosine 5-triphosphate (disodium salt) from Sigma, St. Louis. Creatine phosphate (disodium salt) was purchased from Boehringer, Mannheim. Glucose, glycogen, TRIS, EDTA and other chemicals used in this study were produced by Merck, Darmstadt.

### 3.6.4 Fatty acids

Tissue concentrations of fatty acids were determined according to Van der Vusse et al (1980). Aliquots of deeply frozen tissue (150-300 mg of wet weight) were pulverized in an aluminium mortar with a stainless steel pestle, previously cooled in liquid nitrogen. The tissue powder was transferred to test-tubes cooled with liquid nitrogen. The test-tubes were placed at $-21^{\circ}C$ and the tissue powder was wetted with 2 ml methanol of $-21^{\circ}C$. The content of the test tubes was allowed to warm up till room temperature and was subsequently weighed. Chloroform was added till a mixture of chloroform and methanol of 2:1 v/v was obtained. As antioxydant butylated hydroxytoluene (0.01%) was present in the methanol and chloroform. Subsequently, a mixture of heptadecanoic acid, cholesteryl heptadecanoate and triheptadecanoin was added to the extraction mixture in order to correct for losses during the assay procedure. Non-esterified fatty acids (NEFA), triacylglycerol, cholesteryl esters and total phospholipids were isolated from the extracts by thin-layer chromatography using TLC plates coated with Silica gel F 254. The lipid spots were predeveloped with chloroform/methanol/$H_2O$/acetic acid (10:10:1:1, v/v) until the liquid front had reached a level of 1 cm above the site of application of these spots. Hexane/diethyl ether/acetic acid (24:5:0.3, v/v) was used as developing solvent. The lipid spots were made visible with Rhodamine G, scraped from the plate and transferred into test-tubes, containing 0.5 ml 7% $BF_3$ solution. The fatty acid moiety of the various lipid classes was methylated at $20^{\circ}C$ for 15 min (NEFA) at $100^{\circ}C$ for 30 min (triacylglycerol) and at $100^{\circ}C$ for 45 min (cholesteryl esters and phospholipids). The methylesters were extracted from the methylating mixture with pentane. After evaporation of the pentane under a stream of $N_2$ at $37^{\circ}C$, the methylesters originating from the NEFA, triacylglycerol and phospholipids were dissolved in trimethyl pentane, containing appropriate amounts of methyl pentadecanoate (about 3 nmol per assay) as internal standard. The lipid spots were extracted from the silica gel powder with diethyl ether/methanol (50:1, v/v). The methylester mixtures were analyzed by gas-liquid chromatography using glass columns packed with 5% DEGS on chromosorb W-AWDMCS (length 6 feet; inner diameter 2 mm). Starting temperature of the column was routinely $150^{\circ}C$, the temperature gradually increased up to $190^{\circ}C$, with an increase rate of $4^{\circ}C.min^{-1}$. Carrier flow of $N_2$ was 45 $ml.min^{-1}$.

Tissue samples in this study weighed between 150 and 300 mg. Given a blank value of 3 nmol and NEFA tissue contents of 30 $nmol.g^{-1}$, this implies that

measurements were about three times the blank value. The correction for the blank value was rather accurate in our hands, as is shown by the NEFA intra-assay coefficient of variation of 2.3% (table 3.8).

Also the intra-assay coefficient of other fatty acids in tissue is shown in table 3.8. The exact intra-assay variation of the phosphoglyceride determination can only be validly calculated if the recovery of each individual phosphoglyceride is known. Because this is not the case in this study, the coefficient of variation of the values of two samples in the perfusion area of the left circumflex coronary artery is shown. The real coefficient of variation will be lower or equal to this figure. In table 3.9, the intra-assay coefficient of variation of the individual NEFA in normoxic tissue is shown. Mean coefficient of variation for the different NEFA ranges from 4.8 to 6.1% for the NEFA, present in the relatively higher amount, and from 16.2 to 27.1% for myristic acid, palmitoleic and arachidonic acid, NEFA which are present in very low amounts during normoxia.

Materials
Silicagel F 254, chloroform and methanol were purchased from Merck, Darmstadt, borontrifluorid from Sigma, St. Louis and diethylether, pentane, petroleum-ether ($35^0$-$60^0$C), tri-methyl-pentane and toluene from Baker, Philipsburg.

3.7 Assessment of myocardial metabolism

In the present study, myocardial metabolism was assessed by measuring concentrations of substances in tissue as well as by measuring the arterio-local venous (AV) difference of substances across the normoxic or ischemic myocardium. Division of tissue samples from the outer, middle and inner layers (each about 1/3 of the wall), enabled the study of differences in these processes across the myocardial wall.

Tissue contents of ATP and creatine phosphate were determined to investigate the depletion of high-energy phosphate stores in the ischemic myocardium. AV differences of inorganic phosphate most likely provide information about the release of phosphate liberated from ATP and creatine phosphate into the ischemic venous blood. Determination of the tissue concentrations of breakdown products of high-energy phosphates was not performed because our primary interest was the presence of ATP and creatine phosphate and not the

metabolism of adenine nucleotides.

Substrate metabolism during normoxia as well as ischemia was assessed by measuring tissue glycogen and fatty acid content as well as AV differences for glucose, lactate, NEFA and triacylglycerol. Aerobic metabolism was assessed by measuring the oxygen AV differences.

The influence of ischemia on cellular regulation of ion concentrations was assessed by measuring the AV differences for potassium.

Possible damage of cellular membranes as well as accumulation of possibly noxious substances was estimated by measurement of the tissue content of phosphoglycerides, NEFA and triacylglycerol together with the AV differences of the latter two substances. Gaschromatographic determination of these substances enabled the measurement of the concentrations of the individual fatty acids within these three classes. Because each of these classes has a different relative fatty acid composition in blood and in tissue, accumulation or breakdown from one class into another can be estimated.

Uptake or release of substances by the myocardium can be approximated by the product of total myocardial blood flow and the whole blood (oxygen) or serum (all other substances in the present study) AV difference.

Critique on the experimental design

The major problems inherent to the experimental design chosen, lie in the validity of the assessment of metabolic changes in the ischemic tissue by the uptake or release of substances. Objections include the following: (a) the uptake as determined in this study does not fully cover the utilization since the use of substances released from endogenous stores is not taken into account. Therefore, the term "net uptake" or "net release" is used in the present study; (b) multiplying myocardial blood flow with serum AV differences is only justified if it is assumed that transport of the substances from the plasma into the erythrocyte and vice versa, was not influenced by the inducement of ischemia (chapters 6,7,8) or the substances administered to elevate the arterial NEFA concentrations (chapter 8); (c) another assumption in this calculation is that local venous blood is sampled from the ischemic area in which myocardial blood flow is determined with the radioactive microspheres, without contribution of blood from other, normally perfused areas. However, comparison of the net uptake or release of the various substrates assayed on simultaneously taken blood samples overcomes this critique; (d) during ischemia, net uptake has also to be used with caution, since it is not

clear whether the venous blood samples reflect the average of transmural venous blood or mainly blood from one particular layer.

Critical comments should also be made with regard to the assessment of fatty acid metabolism. AV differences of triacylglycerol can be determined with an accuracy of 3%. However, uptake of triacylglycerol within the error of the determination already has extensive consequences for substrate supply. For the same reason, highly significant increases in the tissue content of NEFA might not necessarily be reflected by reduced contents of triacylglycerol or phosphoglycerides because of the extremely low tissue concentration of the NEFA.

## 3.8 Relation between blood flow, metabolism and mechanics

A major purpose of this study was to relate the changes in myocardial blood flow after inducement of ischemia to the changes in metabolism and mechanics in various layers of the ischemic area. These relations were assessed as follows. Tissue samples for determination of ATP, creatine phosphate, glycogen and fatty acids were taken from the area where the coils for determination of epicardial deformation had been attached. These coils were removed just before taking the biopsies. Because the biopsies were too small for reliable blood flow determination, we have used the blood flow data of the area closely surrounding the tissue used for chemical analysis. The tissue samples for high-energy phosphate and glycogen determination had a diameter of 4 mm, the biopsy for fatty acid analysis had a diameter of 10 mm (in each experiment two of these biopsies were taken from the ischemic area), the samples for determination of myocardial blood flow had a surface of $2$-$4$ cm$^2$ and the surface of the surrounding rectangle of the three coils was $4$-$6$ cm$^2$. This means that the site of sampling of the data concerning blood flow, metabolism and mechanics were not identical. Because of possible inhomogeneities of one or more of these parameters within the same layer of the myocardium, the variation in the mutual relations might be larger than in case of determining all parameters in the same sample. This is also the case for the relation between tissue contents of ATP and NEFA (chapter 7). Information about the size of this variation is given in chapter 4.

4. INVESTIGATION ON THE HOMOGENEITY IN TIME AND SPACE OF MYOCARDIAL MECHANI-
CAL PERFORMANCE, BLOOD FLOW AND METABOLISM IN THE NORMALLY PERFUSED LEFT
VENTRICLE: EVALUATION OF THE EXPERIMENTAL MODEL[*]

## 4.1. Introduction

In the following chapters, the data related to the influence of ischemia
on left ventricular blood flow, metabolism and mechanical performance are
described. The influence of partial occlusion (stenosis) of the left anterior
interventricular coronary artery (LAICA) is evaluated by comparing the diffe-
rences between either the values of the parameters during ischemia with those
before inducement of the stenosis or the values of the parameters in ischemic
and non-ischemic areas of the left ventricle. These comparisons are only
allowed if during the experimental period (2 hours), without inducement of
stenosis, (a) the values of the measured parameters do not change signifi-
cantly and (b) no significant differences exist between the parameters de-
termined in different perfusion areas of the left ventricle.

ad (a). To check the stability of the preparation, two series of experiments
were performed. In the first series we measured hemodynamic vari-
ables, myocardial blood flow (MBF), epicardial deformation, and arte-
rial and local venous concentrations of the substances mentioned in
section 3.6. In the second series, beside these variables also the
tissue contents of ATP, creatine phosphate and glycogen were deter-
mined in biopsies from the myocardium. Results from these investi-
gations are presented in section 4.2.

ad (b). During normoxia, the tissue contents of ATP, creatine phosphate and
glycogen in the perfusion areas of the LAICA and the left circumflex
coronary artery (LCCA) were assessed by taking biopsies from these
areas at various time intervals. Inhomogeneities in MBF, if any, were

[*] Parts of the results presented in this chapter have been published in:
Basic Res. Cardiol. 76: 431-437.

Fig 4.1  Protocol of the experiments.

Table 4.1 Time course of hemodynamic variables during 135 min normoxia
in experiments without (group I, n=10) and with tissue
sampling (biopsies; group II; n=10). Median values and 95%
limits are shown.

|  | group I | | group II | |
|---|---|---|---|---|
|  | -15 min | 120 min | -15 min | 120 min |
| Heart rate ($min^{-1}$) | 90<br>75-155 | 123<br>65-195 | 105<br>65-150 | 123<br>80-140 |
| Systolic aortic pressure (kPa) | 11.9<br>10.4-14.7 | 11.1<br>10.0-13.3 | 12.5<br>11.1-14.7 | 11.7<br>10.9-13.3 |
| Diastolic aortic pressure (kPa) | 8.8<br>7.3-9.3 | 8.6<br>7.7-11.3 | 9.3<br>8.3-10.7 | 9.3<br>8.0-10.3 |
| Systolic coronary artery pressure (kPa) | 12.0<br>10.0-14.7 | 10.7<br>9.3-13.3 | 12.7<br>10.9-14.7 | 11.3<br>10.5-12.8 |
| Diastolic coronary artery pressure (kPa) | 7.6<br>6.7-10.0 | 8.0<br>6.4-9.3 | 8.9<br>6.0-10.0 | 8.7<br>6.7-9.6 |
| $dp_{lv}/dtmax$ ($kPa.s^{-1}$) | 213<br>187-227 | 193<br>160-227 | 213<br>133-240 | 187*<br>133-200 |
| $p_{lved}$ (kPa) | 0.8<br>0.5-1.1 | 0.8<br>0.7-1.2 | 1.1<br>0.5-1.3 | 0.8<br>0.4-1.7 |

* Significantly different from time -15 min.

studied by analyzing the data obtained from various areas of the left
ventricle during the pre-ischemic period. The results of these stu-
dies are presented in section 4.3.

## 4.2 Stability of the preparation during two hours of normal perfusion

### 4.2.1. Protocol

The experiments were performed on mongrel dogs, two groups of 10 animals
each. The protocols are shown in fig 4.1. In group I (without tissue sam-
pling), arterial and local venous samples for blood chemistry were taken at
times -15, 0, 10, 20, 30, 45, 60, 90 and 120 min. The continuously recorded
hemodynamic and local mechanical variables were also determined at these
moments. Regional myocardial blood flow was determined by microsphere injec-
tions at approximately -15, 10, 60 and 120 min. In group II (including tissue
sampling), arterial and local venous blood samples were drawn at times -15,
10, 60 and 120 min. At the same time intervals, cardiac output and the hemo-
dynamic and local mechanical variables were determined. Microspheres were
injected just after -15, 10, 60 and 120 min. Transmural biopsies were taken
from the area perfused by the LAICA a few minutes after the microsphere
injections at times -15, 60 and 120 min and from the area perfused by the
LCCA at times -15 and 120 min.

To study possible changes during the period of 135 min, the values of all
variables determined after sample time -15 min were compared with sample time
120 min. Differences between the values of the various variables were evalua-
ted for statistical significance by applying Wilcoxon's matched-pairs signed-
ranks test.

### 4.2.2 Results

#### 4.2.2.1 Hemodynamic variables

In table 4.1, only the hemodynamic variables in the two groups at times
-15 and 120 min are shown because these variables showed a regular time
course. In both groups heart rate tended to increase in the course of the
experiment, from about 100 to 120 beats.min$^{-1}$, while aortic pressure decrea-
sed by about 0.8 kPa. This decrease was significant at time 120 min in the
experiments where biopsies were taken (group II). Because no stenosis had
been induced, the changes in coronary artery pressure were similar to those

Table 4.2 Arterial concentrations of several chemical variables in the blood at the onset and the end of normoxic experiments without (group I, n=10) and with tissue sampling (group II; n=10). Median values and 95% limits are shown.

|  | group I | | group II | |
|---|---|---|---|---|
|  | -15 min | 120 min | -15 min | 120 min |
| pH | 7.41 | 7.40 | 7.40 | 7.43* |
|  | 7.35-7.45 | 7.38-7.45 | 7.34-7.43 | 7.39-7.50 |
| $P_{CO2}$ (kPa) | 4.1 | 3.9 | 4.3 | 4.1* |
|  | 3.7-4.5 | 3.6-4.4 | 3.6-4.9 | 3.3-4.6 |
| Hb (mmol.$l^{-1}$) | 8.2 | 8.1 | 8.2 | 8.1 |
|  | 6.2-8.8 | 6.3-9.8 | 6.0-9.4 | 6.3-9.1 |
| Oxygen saturation (%) | 0.86 | 0.90 | 1.00 | 0.99 |
|  | 0.82-0.91 | 0.88-0.91 | 0.89-1.01 | 0.92-1.01 |
| Glucose (mmol.$l^{-1}$) | 6.6 | 6.7 | 5.4 | 5.8 |
|  | 5.6-7.8 | 5.5-8.2 | 4.9-8.1 | 4.0-6.0 |
| NEFA (mmol.$l^{-1}$) | 0.21 | 0.20 | 0.28 | 0.30 |
|  | 0.16-0.27 | 0.18-0.32 | 0.10-0.43 | 0.11-0.73 |
| Lactate (mmol.$l^{-1}$) | 1.26 | 1.23 | 2.01 | 1.80 |
|  | 1.05-1.56 | 0.84-1.57 | 1.08-3.53 | 1.51-3.67 |

Table 4.3 Arterial-local venous (AV) differences of several chemical variables in the blood at the onset and the end of normoxic experiments (see table 4.2).

|  | group I | | group II | |
|---|---|---|---|---|
|  | -15 min | 120 min | -15 min | 120 min |
| pH | 0.04 | 0.04 | 0.04 | 0.03 |
|  | 0.02-0.04 | 0.02-0.10 | 0.03-0.07 | 0.02-0.08 |
| Oxygen (mmol.$l^{-1}$) | 4.4 | 4.3 | 4.7 | 4.9 |
|  | 3.5-7.0 | 3.7-6.8 | 4.2-4.9 | 3.0-6.0 |
| Glucose (mmol.$l^{-1}$) | 0.4 | 0.5 | 0.3 | 0.3 |
|  | (-0.3)-0.8 | (-0.1)-1.4 | 0.0-0.6 | (-0.1)-2.2 |
| NEFA (mmol.$l^{-1}$) | 0.06 | 0.05 | 0.04 | 0.06 |
|  | 0.0-0.13 | 0.01-0.13 | 0.00-0.23 | 0.03-0.10 |
| Lactate (mmol.$l^{-1}$) | 0.46 | 0.39 | 0.69 | 0.48 |
|  | (-0.03)-0.55 | 0.13-0.53 | 0.31-0.99 | 0.13-0.71 |
| $K^+$ (mmol.$l^{-1}$) | -0.08 | -0.05 | -0.03 | -0.03 |
|  | (-0.25)-0.05 | (-0.25)-0.10 | (-0.20)-0.05 | (-0.20)-0.10 |
| Inorganic phosphate (mmol.$l^{-1}$) | -0.10 | -0.07 | -0.04 | -0.02 |
|  | (-0.14)-(-0.03) | (-0.10)-0.03 | (-0.09)-0.04 | (-0.04)-0.04 |

* Significantly different from time -15 min

in aortic pressure. The first positive derivative of left ventricular pressure ($dp_{lv}$/dtmax) did not significantly change in the experiments of group I. However, when biopsies were taken (group II), this variable decreased from 213 $kPa.s^{-1}$ at time -15 min to 187 $kPa.s^{-1}$ at time 120 min. In both groups, end-diastolic left ventricular pressure remained almost constant at 0.5-1.7 kPa (95% limits).

### 4.2.2.2 Biochemical variables in blood

The arterial values of pH, $p_{CO2}$ and $O_2$ saturation and the arterial concentrations of Hb, glucose, lactate and non-esterified fatty acids (NEFA) at times -15 and 120 min are shown in table 4.2. Except for a slight but significant increase in pH (from 7.40 to 7.43) and a decrease in $p_{CO2}$ (from 4.3 to 4.1 kPa) in group II (including tissue sampling) no significant differences were observed in both groups. In table 4.3, the AV differences of pH, $p_{CO2}$, $O_2$, glucose, NEFA, lactate, $K^+$ and inorganic phosphate at times -15 and 120 min are shown. No significant changes in these variables could be detected during the experiments in both groups.

### 4.2.2.3 Biochemical variables in myocardial tissue

Changes in tissue contents of ATP, creatine phosphate and glycogen during the experimental period were investigated in the LAICA and LCCA perfusion areas both, in the inner and outer layers separately. Tissue contents of the two energy-rich phosphates did not significantly change in any perfusion area or layer. Glycogen contents in the LAICA area tended to increase within that period (from 120 to 175 $\mu mol.g^{-1}$ dry weight) while these contents tended to decrease in the LCCA area (from 185 to 165 $\mu mol.g^{-1}$ dry weight) (p-values between 0.05 and 0.20). Contents of these substances in the inner and outer layers did not change significantly during the experimental period. To summarize the results, the data of all biopsies were grouped per sample time (table 4.4). Also in this case, no significant changes were observed during the two hour-period.

### 4.2.2.4 Mechanical variables

The values of the epicardial deformation variables are shown in table 4.5. In group I (without tissue sampling), no significant differences were found in each of the variables throughout the experimental period. However, in group II (including tissue sampling), significant decreases were found in

Table 4.4 Values of tissue ATP, CP and glycogen contents ($\mu$mol.g$^{-1}$ dry weight) at the onset and the end of 135 min of normoxia. All transmural samples, and samples from the inner and outer layers were grouped per sample time.

|  | time (min) | median value | 95% limits | $\underline{n}$ |
|---|---|---|---|---|
| ATP | -15 | 17 | 13-21 | 25 |
|  | 120 | 17 | 12-19 | 28 |
| Creatine | -15 | 42 | 35-50 | 22 |
| phosphate | 120 | 42 | 30-51 | 23 |
| Glycogen | -15 | 175 | 130-190 | 29 |
|  | 120 | 165 | 145-190 | 29 |

Table 4.5 Time course of mechanical variables measured by epicardial deformation during 135 min of normoxia without (group I; n=10) and with tissue sampling (group II; n=10).

| Group I | -15 min | 20 min | 60 min | 120 min |
|---|---|---|---|---|
| Circumferential | 6.5 | 6.0 | 6.5 | 5.0 |
| shortening (%) | 3.0-7.0 | 4.0-8.0 | 1.5-10.0 | 1.5-9.0 |
| Base-to-apex | 2.0 | 1.5 | 2.0 | 1.5 |
| shortening (%) | 0.0-4.0 | (-2.0)-4.0 | (-1.0)-3.0 | 0.0-4.0 |
| Shear angle | 0.05 | 0.04 | 0.04 | 0.04 |
| (rad) | 0.02-0.05 | 0.02-0.07 | 0.01-0.05 | 0-0.04 |
| Group II | -15 min | 20 min | 60 min | 120 min |
| Circumferential | 4.1 | 4.3 | 3.4 | 3.4 |
| shortening (%) | 1.7-7.7 | 0.1-8.1 | 0.4-8.1 | 0.9-7.2 |
| Base-to-apex | 5.1 | 1.7* | 1.7* | 0.9* |
| shortening (%) | 0.0-6.8 | 0.0-3.0 | (-0.8)-3.7 | (-0.9)-1.7 |
| Shear angle | 0.04 | 0.05 | 0.05 | 0* |
| (rad) | 0.02-0.12 | 0.01-0.145 | 0.03-0.07 | 0.0-0.14 |

* significantly different from time -15 min

base-to-apex shortening (from 5.1% at -15 min to 0.9% at 120 min) and shear angle (from 0.04 rad at -15 min to 0 rad at 120 min). Circumferential shortening was not significantly depressed (table 4.5).

## 4.2.2.5 Myocardial blood flow

In experiments without tissue sampling (group I), MBF hardly changed during the observation period of 120 min (table 4.6). At that time, only a significant decrease was observed in epicardial MBF in the area perfused by the LCCA and hence an increase in endo/epi ratio. In contrast, in tissue located in the direct vicinity of the site of sampling (group II), MBF in the various layers decreased at time 60 min (data not shown) as well as time 120 min by 5-27%. The most pronounced decrease was seen in the LAICA perfusion area. In the area perfused by the LCCA, MBF was not significantly affected. In the LAICA perfusion area, the decrease in the various layers amounted to 11-26% ($p < 0.05$; table 4.6).

## 4.2.3 Discussion

The tendency of increasing heart rate during the two hour experimental period can be explained by the diminished activity of fentanyl, given in the premedication, which is known to be a vagotropic compound. The decrease in blood pressure likely results from the barbiturate infusion.

Except for the above-mentioned changes, no significant deviations occurred in the hemodynamic variables and myocardial mechanics, metabolism and blood flow if no biopsies are taken during the experiment. In contrast, significant decreases in $dp_{lv}/dtmax$, base-to-apex shortening and phase angle are seen when tissue samples are taken within the experimental period. Yet MBF decreases in the direct vicinity of the site of sampling and even in the whole LAICA perfusion area. This is in agreement with the results of Allard et al (1981) who found, taking biopsies of similar diameter as compared with the present study (4 mm), a decrease in MBF around biopsy sites from 30% within 0.5 cm from that site to 15% at 2-3 cm. This shows that by taking biopsies a rather extensive part of the left ventricular free wall is affected.

Beside these disadvantages of taking biopsies during the experiment, another drawback should be mentioned. In 3 out of 11 experiments ventricular fibrillation occurred already after taking the first biopsy. In 2 of these cases, no recovery occurred.

Because of these findings, the protocol of the experiments in which ische-

Table 4.6

Values of regional myocardial blood flow at the onset and the end of 135 min of normoxia in the LCCA and LAICA perfusion area during experiments without (group I) and with tissue sampling (group II). In the latter case, data are shown of tissue samples immediately surrounding the biopsy holes as well as of the whole perfusion area.

| | group I | | group II | | | |
| | | | around biopsy holes | | whole perfusion area | |
| | -15 min | 120 min | -15 min | 120 min | -15 min | 120 min |
|---|---|---|---|---|---|---|
| **LAICA area** | | | | | | |
| epi | 0.67 0.20-0.82 | 0.56 0.31-0.70 | 0.74 0.44-1.05 | 0.47 0.36-0.87* | 0.73 0.37-0.97 | 0.54 0.31-0.93* |
| meso | 0.54 0.34-0.73 | 0.46 0.26-0.72 | 0.54 0.42-0.84 | 0.47 0.25-0.78 | 0.52 0.38-0.64 | 0.44 0.33-0.64* |
| endo | 0.50 0.34-0.61 | 0.39 0.25-0.57 | 0.58 0.44-0.73 | 0.43 0.24-0.63* | 0.48 0.42-0.65 | 0.43 0.32-0.50* |
| endo/epi ratio | 0.79 0.56-1.20 | 0.69 0.51-1.29 | 0.75 0.69-0.99 | 0.73 0.53-0.88 | 0.74 0.60-1.11 | 0.76 0.62-0.93 |
| mean MBF | – – | – – | – – | – – | 0.59 0.39-0.77 | 0.48 0.35-0.73* |
| **LCCA area** | | | | | | |
| epi | 0.52 0.33-0.64 | 0.41 0.28-0.60* | 0.73 0.43-0.95 | 0.57 0.42-0.86* | 0.55 0.34-0.81 | 0.53 0.44-0.80 |
| meso | 0.53 0.45-0.72 | 0.54 0.39-0.68 | 0.67 0.42-0.89 | 0.58 0.45-0.73 | 0.65 0.47-0.91 | 0.60 0.53-0.75 |
| endo | 0.56 0.41-0.74 | 0.51 0.36-0.62 | 0.62 0.41-0.85 | 0.59 0.49-0.72 | 0.65 0.47-0.87 | 0.59 0.57-0.79 |
| endo/epi ratio | 1.15 0.94-1.17 | 1.23 1.06-1.51* | 0.96 0.77-1.18 | 1.11 0.85-1.21 | 1.17 1.06-1.39 | 1.20 0.93-1.33 |
| mean MBF | – – | – – | – – | – – | 0.62 0.42-0.86 | 0.57 0.52-0.76 |

mia was induced was changed and it was decided to stop each experiment after two tissue samples were taken; one from the area perfused by the LAICA and one from the area perfused by the LCCA. In this way, tissue contents of ATP, creatine phosphate and glycogen at various time intervals after inducement of the stenosis were obtained from different groups of animals (see chapter 6 and 7).

## 4.3 Regional differences in left ventricular blood flow and metabolism during normal perfusion

### 4.3.1 Protocol

#### 4.3.1.1 Biochemical variables in myocardial tissue

To investigate whether differences in tissue contents of ATP, creatine phosphate and glycogen exist between different layers of the myocardium, the contents of samples in the inner layers were compared with those from the outer layers within each experiment. Similarly, the contents of the three substances in the areas perfused by the LAICA and the LCCA were evaluated on differences, if any. For this purpose we used the data presented in section 4.2. Differences between the value of the various vatriables were evaluated for statistical significance by applying Wilcoxon's matched pairs signed-ranks test (two tailed probability).

#### 4.3.1.2 Myocardial blood flow

To study possible inhomogeneities in axial and circumferential direction, MBF and the endo/epicardial flow ratios (endo/epi ratio) were grouped per slice (1-5) and per section (A-E), (fig 3.8). Adding the MBF values and the endo/epi ratios of all pieces provided respectively total MBF and the overall endo/epi ratio.

Differences in MBF between various sites of the free wall of the left ventricle were evaluated for statistical significance by applying a two-way variance analysis for the MBF per layer and a one-way analysis for the endo/-epi ratios. A p value<0.05 was considered to be significant.

Table 4.7 The content of ATP, creatine phosphate and glycogen ($\mu$mol.-g$^{-1}$ dry weight) per perfusion area (LAICA and LCCA) as derived from all normoxic tissue samples (see text).

|  | perfusion area | median value | 95% limits | n |
|---|---|---|---|---|
| ATP | LAICA | 19* | 16-26 | 23 |
|  | LCCA | 18 | 13-19 | 23 |
| Creatine | LAICA | 45 | 38-54 | 21 |
| phosphate | LCCA | 41 | 35-51 | 21 |
| Glycogen | LAICA | 165 | 135-190 | 24 |
|  | LCCA | 165 | 150-185 | 24 |

* $p < 0.05$ as compared with the LCCA area

Table 4.8 Transmural contents of ATP, creatine phosphate and glycogen ($\mu$mol.g$^{-1}$ dry weight) during normoxia. All tissue samples have been grouped per location: outer layers=epi and inner layers=endo.

|  | Location | Median value | 95% limits | n |
|---|---|---|---|---|
| ATP | epi | 21 | 17-26 | 31 |
|  | endo | 17 | 13-20 | 31 |
| Creatine | epi | 45 | 40-57 | 29 |
| phosphate | endo | 41 | 34-54 | 29 |
| Glycogen | epi | 165 | 145-205 | 33 |
|  | endo | 165 | 150-180 | 33 |



Fig 4.2
Myocardial blood flow in the outer (epi, ▨ ) and inner layers (endo, □ ) and the ratio of blood flow in the inner and outer layers (endo/epi ratio, ■) in the sections A-E of the left ventricular free wall. Characters above each bar indicate significant differences between the particular section and the indicated one(s). The means and standard deviation of 17 experiments are presented.

## 4.3.2 Results

### 4.3.2.1 Biochemical variables in myocardial tissue

As can be seen from table 4.7, no significant differences in ATP, creatine phosphate or glycogen content were observed between biopsies from the LAICA and LCCA area, in the inner layers, the outer layers or the transmural biopsies, except for the ATP content in samples from the outer layers of the LAICA area which was significantly higher than that in similar biopsies from the LCCA area. The transmural differences in the three substances were not significant, although the median values of ATP and creatine phosphate were higher in the epicardium (table 4.8).

### 4.3.2.2 Myocardial blood flow

Overall MBF in the left ventricular free wall was $0.64 \pm 0.23$ ml.min.$^{-1}$.-$g^{-1}$ (mean $\pm$ s.d.). The overall endo/epi ratio in this part of the myocardium was $1.05 \pm 0.31$ (mean $\pm$ s.d.). The mean values of blood flow in the inner and outer myocardial layers and of the endo/epi ratios per sample site are shown in table 4.9. The mean values of these variables per slice and per section are shown in the figs. 4.2 and 4.3, respectively. The values of blood flow in the outer layers were the lowest at the base of the left ventricle, near the marginal branch (E1) and increased gradually in the direction of the apex and of the LAICA. Blood flow in the outer layers was lower near the base than near the apex of the heart and higher along the LAICA than along the marginal branch. In the middle layer, MBF was almost equally distributed over the left ventricular free wall. The blood flow values in the inner layers although less pronounced, tended to be opposite to the values in the outer layers. The endo/epi ratios were higher near the base than near the apex, and higher along the marginal branch than along the LAICA. The highest mean value (1.44) was found at the base of the heart near the marginal branch.

## 4.3.3 Discussion

### 4.3.3.1 Biochemical variables in myocardial tissue

It is known that the ATP and especially the creatine phosphate contents rapidly decrease in biopsies between sampling and freezing. Kleinert and Weiss (1981) showed that the biopsies should be frozen within 5 s while results of Hearse and Chappell (1976) suggest the necessity of an even shor-

Table 4.9 Distribution of blood flow in the epi (upper) and endo-cardial layers (middle) and of the endo/epicardial blood flow ratio (lower panel) in the left ventricular free wall. The mean values of 17 experiments are presented.

| | | A | B | C | D | E |
|---|---|---|---|---|---|---|
| epi | 1 | 0.64 | 0.67 | 0.58 | 0.55 | 0.50 |
| | 2 | 0.64 | 0.71 | 0.63 | 0.61 | 0.52 |
| | 3 | 0.67 | 0.68 | 0.63 | 0.63 | 0.56 |
| | 4 | 0.69 | 0.72 | 0.62 | 0.67 | 0.62 |
| | 5 | 0.75 | 0.77 | 0.85 | 0.77 | 0.73 |
| endo | 1 | 0.68 | 0.61 | 0.60 | 0.62 | 0.67 |
| | 2 | 0.60 | 0.64 | 0.66 | 0.71 | 0.69 |
| | 3 | 0.60 | 0.64 | 0.68 | 0.68 | 0.66 |
| | 4 | 0.58 | 0.63 | 0.64 | 0.64 | 0.61 |
| | 5 | 0.63 | 0.59 | 0.59 | 0.65 | 0.64 |
| ratio | 1 | 1.09 | 0.94 | 1.06 | 1.14 | 1.41 |
| | 2 | 0.99 | 0.93 | 1.08 | 1.21 | 1.44 |
| | 3 | 0.92 | 1.03 | 1.09 | 1.12 | 1.23 |
| | 4 | 0.92 | 0.93 | 1.06 | 1.00 | 1.07 |
| | 5 | 0.88 | 0.81 | 0.78 | 0.97 | 0.98 |

Fig 4.3
Myocardial blood flow in the outer (epi, ▨) and inner layers (endo, □) and the endo/epi ratio (■) in the slices 1-5 of the left ventricular free wall. Figures above each bar indicate significant differences between the particular slice and the indicated one(s). The means and standard deviation of 17 experiments are presented.

ter delay time. In our hands, creatine phosphate contents as determined in biopsies from normoxic tissue more than 5 s after sampling showed a decrease of twice the standard deviation below the mean value of these contents in biopsies taken within 3 s. Therefore, only biopsies were used with a delay time shorter than 3 s. The usual transit time in our preparation was 2-3 s. The normoxic contents of ATP and creatine phosphate as found in the present investigation were 18 (15-20) and 42 (36-45) $\mu mol.g^{-1}$ dry weight, respectively (median values and 95% limits). These values are comparable with values from other investigators (ATP 15-22 and creatine phosphate: 30-50 $\mu mol.g^{-1}$ dry weight (Takenaka and Higuchi, 1974; Hearse and Chappell, 1976; Opie and Owen, 1976; Weishaar et al, 1977; Allison et al, 1978; Dunn et al, 1978). Our data are in close agreement with those obtained by Allard and co-workers (1981). These authors used a rapid freezing biopsy drill drawing the biopsy into the liquid nitrogen within 1.75 s by vacuum and found creatine phosphate values of 40 and ATP values of $18 \mu mol.g^{-1}$ dry weight.

The absence of significant differences between ATP contents of the inner and outer myocardial layers is equivocal with data from the literature (Takenaka and Higuchi, 1974; Dunn et al, 1978; Deboer et al, 1980).

Significantly lower creatine phosphate contents in the subendocardium were found by Dunn et al (1978) and Takenaka and Higuchi (1974). Although the difference between layers was not significant in the present study, the 10% lower median value in the inner than in the outer layer is equal to the difference found by Dunn et al (1978) who performed a substantial larger number of experiments.

In the present study, glycogen contents of 165 (130-190) $\mu mol. g^{-1}$ dry weight have been measured (median value and 95% limits). Glycogen contents found in the normoxic dog myocardium in situ range from 100 to 250 $\mu mol.g^{-1}$ dry weight (Jedeikin, 1964 and Crass I and Sterrett, 1975, respectively). The values found in our study are comparable with the more recent findings which show values of 150-250 $\mu mol.g^{-1}$ dry weight (Hewitt et al, 1973; Crass and Sterrett, 1975, Allison et al, 1978, Ichihara et al, 1979).

Concerning the transmural distribution of glycogen conflicting results have been presented. Opie et al (1976) did not find transmural differences in the baboon, while Ichihara and Abiko (1975), Allison et al (1977), Jedeikin (1964) and Crass and Sterrett (1975) found higher endocardial than epicardial contents of glycogen in the dog heart. However, the latter two authors showed that this high glycogen content is only present in the cells of the His

bundle and Purkinje system. A different way of dividing the biopsies into different layers might have caused the absence of a significantly higher glycogen content in the inner layers than in the outer ones.

In our preparation, little differences are present between the tissue contents of the various substances in the LAICA and LCCA perfusion area, even when various layers of the myocardium were compared. This was the case for creatine phosphate and glycogen (table 4.7). The small but significant difference in ATP contents between the two perfusion areas seems of little physiological significance. From these findings, it was concluded that the tissue contents in the area perfused by the LCCA are representative of the normoxic content of the free wall. This enables us to make intra-individual comparisons between ATP, creatine phosphate and glycogen contents in normoxic myocardium (LCCA sample) and their content in ischemic myocardium (LAICA sample) in the experiments where the LAICA was partially occluded. Moreover, it will be shown that the tissue concentrations of these three substances do not significantly change in the LCCA area within 2 hours after inducement of a stenosis of the LAICA (chapter 7).

## 4.3.3.2. Myocardial blood flow

The present study indicates that the endo/epi ratios are higher near the base than near the apex of the heart (axial gradient) and lower along the LAICA than along the marginal branch of the LCCA (circumferential gradient). The axial gradient in endo/epi ratios confirms the finding of Reneman et al (1977) and Marcus et al (1975), while the circumferential gradient in this variable is in agreement with the findings in other studies in open- and closed-chest animals (Cobb et al, 1974; Prokop et al, 1974; Ball et al, 1975). These gradients mainly result from variations in blood flow in the outer layers which is lower near the base than near the apex and higher along the LAICA than along the marginal branch.

An obvious question is whether the variations in blood flow in the outer layers over the left ventricular free wall are real or due to artefacts. Assuming a direct relation between blood flow and mechanical performance, real inhomogeneities imply differences in workload at various sites of the left ventricular free wall or in case of an equally distributed workload, differences in oxygen extraction from site to site. On the one hand, Weiss and co-investigators did not find significant differences in oxygen uptake over the free wall of the left ventricle (1978) suggesting an equally distri-

buted workload. On the other hand, regional differences in epicardial as well as intramural shortening have been found in open-chest dogs (Ingels et al, 1971; Elshuraydeh, 1981).

Variations in endo/epi ratios might result from the open-chest preparation, used in the present study. Indeed, Cobb et al (1974) showed that MBF was rather homogeneously distributed in the non-anesthetized dog, but that a similar circumferential gradient in endo/epi ratio was found, as in our investigation, when the experiments were performed in open-chest animals. In their study, however, this gradient was mainly caused by variations in MBF in the inner layers, which is in contrast with our study. The findings of Cobb et al (1974) suggest that drying out or shrinking of epicardial tissue in the outer layers in the open-chest preparation cannot be considered as the major cause of the inhomogeneities in blood flow in these layers found in the present study. Besides, shrinking or drying out do neither explain the higher blood flow in the outer layers values along the LAICA than along the marginal branch - the opposite would be expected - nor the differences in this variable from base to apex, because the latter areas are about equally exposed to the open air. An effect of the open-chest preparation which cannot be excluded in advance, is the low temperature in the outer layers of the wall although Ten Velden et al (1982) did not find significant differences in blood flow distribution when epicardial temperatures were decreased by about $3^0C$.

A source for artefacts may be the microsphere assay method. Microspheres, after all, tend to migrate axially within a blood vessel because of their substantial mass (Segre, 1961; Phibbs and Dong, 1970). Overestimation of blood flow in the inner layers has been ascribed to this streaming (Domenech et al, 1969; Yipintsoi et al, 1973; Utley et al, 1974). Streaming of microspheres and relatively low flow velocities in the side branches of the LAICA and of the marginal branch could explain the higher concentration of beads near the apex than near the base. The lower blood flow values in the outer layers along the relatively small marginal branch, than along the LAICA support this idea. On the other hand, streaming does not explain the higher blood flow values along the LAICA in the outer layers than in the inner ones. Part of the described inhomogeneities in blood flow were also found when the $K^{43}$ method was used (Prokop et al, 1974).

It is not likely that ligation of a small side branch of the LAICA, to insert the pressure catheter, is responsible for the lower endo/epicardial ratios near the apex. First of all, the lower ratio is mainly caused by

changes in blood flow in the outer layers. Secondly, in case of ischemia a more pronounced local fall in blood flow in the inner layers would have been expected (Bishop et al, 1976). Moreover, the endocardial blood flow values near the apex as obtained in the present study did not differ significantly from those found in experiments without implanted catheters for coronary artery pressure measurements (unpublished results).

In conclusion, at the present state of knowledge it remains uncertain whether the inhomogeneities in blood flow distribution in the various layers of the left ventricular free wall are real or partially or completely due to artefacts.

## 4.4 Conclusions

- When no biopsies were taken during the experiments, the preparation is stable during 135 min of normal perfusion.
- Experiments where ischemia will be induced, should be terminated after biopsies have been taken. To evaluate the changes due to ischemia, the contents in the biopsies from the ischemic LAICA area can be compared with the contents in the biopsies from the non-ischemic LCCA area in the same experiment.
- Regional blood flow in the outer layers of the left ventricular free wall was higher near the base than near the apex and lower in the posterior than in the anterior regions. The cause of this inhomogeneity is not known.

5. PREDICTION OF FIBER SHORTENING IN THE INNER LAYERS OF THE LEFT VENTRICULAR WALL FROM EPICARDIAL DEFORMATION MEASUREMENTS DURING NORMOXIA AND ISCHEMIA

## 5.1 Introduction

During ischemia steep gradients in myocardial blood flow and metabolic variables indicate that the inner layers of the left ventricular wall are more susceptible to ischemia than the outer layers (chapter 1). Determination of mechanical performance during acute occlusion of a coronary artery showed very rapid loss of contractile function and even indicated lengthening of ischemic segments during systole ("systolic bulging") (Tennant and Wiggers, 1935; Banka and Helfant, 1974; Tyberg et al, 1974; Theroux et al, 1976; Crozatier et al, 1977).

Little is known about transmural differences in mechanical performance during regional ischemia because of the difficulties encountered in assessing these differences. The most recent approach has been the measurement of transmural shortening by positioning pairs of ultrasonic crystals within the outer and inner layers (Weintraub et al, 1981). This method, however, has disadvantages. Firstly, improper location or movement of the crystals due to the mechanical activity of the ventricle may change the orientation of the crystals with respect to each other, causing inaccurate measurements. Secondly, the positioning of crystals within the myocardium induces injuries because positioning of the crystals in the inner layers requires incisions or stab wounds of about 1 cm deep.

A different approach is to estimate transmural differences in fiber shortening from epicardial deformation. The principle of this method is the assumption, that shortening along the fiber direction in a certain layer in the myocardial wall is closely related to shortening along the same direction at the epicardial surface. Thus shortening of the fibers in the inner and outer layers is estimated from shortening of the epicardial surface along the fiber direction in the inner and outer layers. Epicardial deformation was assessed by an inductive technique (Arts and Reneman, 1980). From thus measured values of circumferential natural strain, base-to-apex natural strain, and shear, shortening along all directions on the epicardial surface could

.

be calculated.

The aim of this part of the study was to investigate the influence of local ischemia on (a) regional mechanical function as measured by epicardial deformation, and (b) on transmural gradients in contractile behavior in the wall of the left ventricle.

In order to assess the validity of the technique of obtaining information about the transmural contractile behavior from measurement of epicardial deformation, 6 additional experiments were performed. In these experiments deformation of the epicardium and the inner layers was assessed simultaneously by measurement of mutual movement and angulation of 3 needles pierced into the myocardial wall, using the inductive technique.

## 5.2 Experimental design

### 5.2.1 Protocol

The experiments were performed on 19 mongrel dogs. The animals were allotted to 2 groups. In group 1, consisting of 13 animals, also used in the studies described in the chapters 6 and 7, epicardial deformation was measured (section 3.1.3 and 3.3.2). From these measurements epicardial maximal shortening ($e_{max}$) and minimal shortening ($e_{min}$) and the angle of $e_{min}$ with the circumference ($\beta_{min}$) were calculated as described in section 3.3.2.2. In the 6 animals of group II, transmural deformation was assessed using the method described in section 3.3.3. In these experiments, from the assessed $e_c$, $e_z$ and $\gamma$ at the epicardium and in the inner layers, $e_{max}$, $e_{min}$ and $\beta_{min}$ at the epicardium and their counterparts in the inner layers ($e_{max,endo}$, $e_{min,endo}$ and $\beta_{min,endo}$, respectively) were calculated. Moreover, shortening in the inner layers in the direction of $e_{min}$, called $e_{endo}$ (section 3.3.3.1). In both groups, ECG, left ventricular pressure, aortic pressure, coronary artery pressure and phasic aortic flow were determined as described in section 3.2.

Following a base-line registration, in all animals the left anterior interventricular coronary artery (LAICA) was occluded by inflation of the occluder cuff around this artery (section 3.1.4). After

Fig 5.1 Protocols of the experiments. Beside the regional mechanical variables, left ventricular, aortic and coronary artery pressure as well as phasic aortic flow were determined at the depicted sample times.



Fig 5.2 Samples of the registration of circumferential shortening $(e_c)$, base-to-apex shortening $(e_z)$, shear angle $(\gamma)$, phasic aortic flow and ECG of a typical experiment. Onset and end of the ejection phase are marked by dots.

one minute, the occlusion was released. In group II a second coronary
artery occlusion of one min was performed. The time interval between
these occlusions was at least 15 min, which was considered to be
sufficient for recovery of the myocardium from the previous occlusion
(fig 5.1).

The values of all continuously registrated variables were calcu-
lated just before and 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 s after
coronary artery occlusion in the epicardial deformation study (group
I) and just before and 5, 10, 20, 40, and 60 s after occlusion in the
transmural deformation study (group II; fig 5.1).

## 5.2.2 Data analysis

Information about the effect of acute coronary artery occlusion on
regional myocardial mechanical performance was obtained by using the
animal as its own control. The effects on the various variables were
evaluated by comparing the values during occlusion with those just
before occlusion (time 0 s). Moreover, changes in these variables
after 20 s of occlusion were evaluated by comparing the data at time
30, 40, 50 and 60 s with those at time 20 s. Differences between the
values of the various variables were evaluated for statistical signi-
ficance by applying Wilcoxon's matched pairs signed-ranks test (two-
tailed probability).

The relationship beween $e_{endo}$ and $e_{min}$ was investigated by linear
correlation and tested for significance by Spearman's signed-rank
correlation test.

The data are presented as median values and 95% limits. A value of
$p < 0.05$ was considered to be a significant difference.

## 5.3 Results

### 5.3.1 Epicardial deformation (group I)

During normal perfusion heart rate and mean aortic pressure were
118 (92-127) beats.$min^{-1}$, and 11.9 (9.6-13.3) kPa, respectively,
(median values and 95% limits). After one minute occlusion, heart rate
increased to 140 (84-160) beats.$min^{-1}$ ($p < 0.05$), while mean aortic
pressure did not change significantly (11.9 ;9.3-12.4 kPa). Mean

Fig 5.3
Time course of $e_c$, $e_z$ and $\gamma$ during one min of acute coronary artery occlusion as determined from epicardial deformation measurements (n=13). The arrow indicates the onset of occlusion.
Median values and 95% limits are shown.
*p<0.05 as compared with t=0 s.
**p<0.05 as compared with t=20 s.

Fig 5.4
Time course of $e_{min}$ and $e_{max}$ during one min of acute coronary artery occlusion as determined from epicardial deformation measurements (n=13). For explanation of the symbols, see fig 5.3.

coronary artery pressure ($p_{cor}$) was 10.5 (9.2-14.0) kPa before coronary artery occlusion. After occlusion, mean $p_{cor}$ decreased significantly to 3.7 (2.5-4.7) kPa after 2 s, 2.9 (2.0-4.3) kPa after 4 s, 2.5 (1.7-3.5) kPa after 6 s, 2.5 (1.5-2.8) kPa after 10 s and 2.7 (2.0-3.3) kPa after 60 s.

An example of the registration of $e_c$, $e_z$, $\gamma$, aortic flow and ECG just before and 5, 10, 20 and 60 s after occlusion is given in fig 5.2. Already after 5 s circumferential lengthening occurred before the end of ventricular ejection, followed by a delayed shortening during the end of the relaxation phase. With prolongation of occlusion, maximum shortening during the ejection phase decreased. After 60 s occlusion, circumferential lengthening occurred already in the isovolumic phase and hardly any changes in length were found during the ejection phase. In this experiment, changes in shear angle were not seen before 20 s of occlusion. Thereafter, a gradual decrease takes place to one third of the pre-occlusion value after 60 s.

Under normal conditions, the median values and 95% limits of circumferential shortening ($e_c$), base-to-apex shortening ($e_z$) and shear angle ($\gamma$) during the ejection phase were 7.4% (5.7-8.7%), 7.7% (3.6-9.4%) and 0.13 (0.08-0.15) rad, respectively (fig 5.3). After 4 s of occlusion, $e_c$ decreased and became negative (=lengthening) after 10 s occlusion. $e_c$ reached its minimum after about 20 s, increasing thereafter to less negative values. A significant decrease in $e_z$ was not observed before 10 sec after onset of occlusion. Moreover, base-to-apex lengthening was only seen in a few experiments. The median value of $e_z$ was close to zero after 20 s and slightly increased thereafter.

In the first 20 s of occlusion, changes in $\gamma$ showed large interindividual variations: an increase was found in 7 experiments, and a decrease in 6 experiments. However within each experiment these early changes in $\gamma$ always preceeded the changes in $e_c$ and $e_z$. From 20 to 60 s after onset of occlusion, $\gamma$ gradually decreased in all experiments to a value about one fifth of the pre-occlusion value (fig 5.3).

The length of the circumferential and base-to-apex segments (distance between the coils) at the onset of the ejection phase increased, starting 20 s after occlusion (table 5.1). This pre-ejection length gradually increased to 107.7 and 107.9% of the pre-ischemic values in

Table 5.1 Time course of circumferential and base-to-apex segment
length (=distance of the coils) at the onset of the ejection
phase before and after acute coronary artery occlusion,
normalized on the segmentlength at time 0 s. (n=13)

| Time (s) | 0 | 10 | 20 | 40 | 60 |
|---|---|---|---|---|---|
| Circumfe-<br>rential(%) | 100.0<br>- | 101.2*<br>100.0-102.1 | 102.1*<br>100.6-103.4 | 106.4*<br>103.4-107.7 | 107.7*<br>106.0-110.2 |
| Base-to-<br>apex(%) | 100.0<br>- | 102.0<br>98.3-106.8 | 104.8*<br>100.9-108.5 | 107.2<br>101.3-116.2 | 107.9*<br>104.3-114.9 |

* = $p < 0.05$ as compared with t 0 s.

Table 5.2 Epicardial and subendocardial $e_{min}$, $e_{max}$, and the direction
of $e_{min}$ referred to the circumference ($\beta_{min}$) as obtained from a ma-
thematical model (Arts et al, 1982a) and from measurement of transmu-
ral deformation (median values and 95% limits, 6 animals, n=12).

|  |  | Model | Measurement |
|---|---|---|---|
| Epicardium | $e_{max}$ (%) | 15 | 15<br>9-19 |
|  | $e_{min}$ (%) | 2 | 3<br>2-6 |
|  | $\beta_{min}$ (rad) | 0.75 | 0.86<br>0.20-1.37 |
| Subendocardium | $e_{max,endo}$ (%) | 28 | 23*<br>10-27 |
|  | $e_{min,endo}$ (%) | 8 | 2*<br>5-8 |
|  | $\beta_{min,endo}$ (rad) | 1.4 | 0.83*<br>0.09-1.45 |

* $p < 0.05$ as compared with the model

Table 5.3 $\beta_{min}$ on the epicardium and in the inner layers ($\beta_{min,endo}$)
before and after acute coronary artery occlusion in 6 ani-
mals (n=12). Median values and 95% limits are shown.

| Time (s) | 0 | 5 | 10 | 20 | 40 | 60 |
|---|---|---|---|---|---|---|
| $\beta_{min}$<br>(rad) | 0.86<br>0.20-1.37 | 1.11<br>0.75-1.45 | 1.07*<br>0.86-1.44 | 1.06*<br>0.90-1.18 | 1.03<br>0.65-1.34 | 0.67<br>0.44-1.36 |
| $\beta_{min,endo}$<br>(rad) | 0.83<br>0.09-1.45 | 1.08<br>0.83-1.31 | 1.30*<br>0.94-1.41 | 0.98*<br>0.90-1.54 | 1.01<br>0.75-1,42 | 0.88<br>0.60-1.48 |

* $p < 0.05$ compared with time 0 s.

circumferential and base-to-apex direction, respectively which means a
15.6% enlargement of the epicardial surface.

The time course of $e_{max}$ and $e_{min}$, as calculated from $e_c$, $e_z$ and
(see above) is shown in fig 5.4. A significant decrease occurred
approximately 2 s earlier in $e_{min}$ than in $e_{max}$. Moreover, values of
$e_{max}$ remained positive whereas values of $e_{min}$ became negative within 8
s of occlusion. A further difference between these two variables is
that the values of $e_{max}$ did not change between 20 and 60 s of occlusi-
on whereas the values of $e_{min}$ increased after 20 s to just below zero.

## 5.3.2 Transmural deformation (group II)

Table 5.2 shows the experimental data on maximum and minimum shor-
tening and the direction $\beta_{min}$ of minimum shortening at the epicardial
surface and in the inner layers, as well as values of these variables
obtained from model calculations (Arts et al, 1982b). The median of
the measured values of epicardial $e_{max}$, $e_{min}$ and $\beta_{min}$ as measured,
appear to be close to the values of these variables as calculated
with the model of left ventricular wall mechanics. The difference
between the measured and predicted value of maximal shortening in the
inner layers ($e_{max,endo}$) was less than 25% but significant. The measu-
red values of both $e_{min}$ and $\beta_{min}$ in the inner layers ($e_{min,endo}$ and
$\beta_{min,endo}$, respectively) were found to be significantly lower than the
model calculations of these variables. Both predicted and measured
values of $e_{min,endo}$ were higher as compared with their epicardial
counterparts ($e_{min}$).

The values of $e_{min}$ and $e_{endo}$ obtained during normoxia and one min
of coronary artery occlusion were plotted against each other for each
individual experiment (fig 5.5). In 5 out of 6 experiments, the rela-
tion between the latter parameters was significant ($p < 0.05$). Based on
the combination of all data the relation between the two parameters
was found to be highly significant ($p < 0.001$).

Fig 5.6 shows the time course of $e_{min}$ and $e_{endo}$ as assessed from
transmural deformation. During the normoxic and ischemic period, $e_{min}$
and $e_{endo}$ were not significantly different. The values of both vari-
ables became negative within 10 s after coronary artery occlusion,
indicating lengthening along the corresponding direction during the

Fig 5.5 Relationship between $e_{min}$ and $e_{endo}$ during normoxia and ischemia in 6 experiments. The encircled figures depict the sample time (s) after onset of acute coronary artery occlusion. Regression equation and correlation coefficient were determined according to linear regression.

ejection phase. Lengthening occurred during the rest of the experimental period.

Before as well as after acute coronary artery occlusion, the orientation of $e_{min,endo}$ and $e_{max,endo}$ was not significantly different from the orientation of their epicardial counterparts (table 5.3). At each sample time, the angle between $e_{min}$ and $e_{endo}$ was less than 0.5 rad. Both angles $\beta_{min}$ showed a transient increase 10 and 20 s after the onset of occlusion.

Both $e_{max}$ on the epicardium and $e_{max,endo}$ in the inner layers decreased after occlusion, the fall in the latter variable being more pronounced in the first 10 s (fig 5.7). However, 60 s after occlusion the relative decrease in shortening is approximately the same in both layers (fig 5.7).


## 5.4 Discussion

### 5.4.1 Reliability of the assessment of the transmural course of fiber shortening from epicardial deformation.

The amount of shortening in the deeper layers of the myocardium can be estimated from epicardial deformation if shear between the various layers in the left ventricular wall is small compared to changes in strain along the fibers during contraction. During normal perfusion, Feigl and Fry (1964) showed the latter shear to be 0.02 rad without preferential direction. These authors concluded that in the normoxic heart the transmural course of deformation during ejection might be determined with reasonable accuracy from the deformation of the epicardial surface.

In the present study, the effect of acute coronary artery occlusion on epicardial and subendocardial deformation was assessed in open-chest dogs,using the inductive technique, mentioned in section 3.3. To assess the effect of shear between the myocardial layers during normoxia and ischemia, in 6 experiments transmural deformation was measured directly, using 3 needles each with a pair of coils attached to it and pierced through the myocardial wall (group II).

Inaccuracy of the latter measuring technique might be introduced by impaired mobility of the measuring needles because of their inertia.

Fig 5.6 Time course of $e_{min}$ (— ■ —) and $e_{endo}$ (··●··) during 1 min of acute coronary artery occlusion as determined from transmural deformation measurements (data from 2 occlusions in 6 animals). For explanation of the symbols, see fig 5.3.



Fig 5.7
Time course of $e_{max}$ (— ■ —) and $e_{max,endo}$ (··●··) during one min of acute coronary artery occlusion, determined from transmural deformation measurements (data from 2 occlusions in 6 animals). Median values and 95% limits are shown.

The influence of inertia on the measurement is probably small because our data were obtained during systole, when the stiffness of the myocardium is increased, and the mechanical contact of the needles with the surrounding tissue is improved. The minor importance of the inertia of the needles with coils is also indicated by the fast responses, found in the deformation signals during the cardiac cycle, up to 21Hz.

Using the above-mentioned technique, no significant differences could be observed between the values of epicardial minimal shortening ($e_{min}$) and shortening in the $e_{min}$ direction in the inner layers ($e_{endo}$) during normoxia as well as ischemia, although possible differences could be masked by the large variation in the values of $e_{endo}$. The variation in $e_{min}$ as determined by epicardial and transmural deformation measurements is similar as shown by comparing the figs 5.4 and 5.6. Except for the influence of shear between the various layers, the greater variation in $e_{endo}$ might result from the fact that $e_{endo}$ is calculated from strains, the values of which are extrapolations from strains measured over the epicardium. Also non-parallel orientation of the needles through the ventricular wall could induce variations in the measurements. In both cases, initially small errors at the levels of the coils will cause larger errors at the level of the subendocardium. Despite this large variation in $e_{endo}$, the relation between $e_{min}$ and $e_{endo}$ was significant (fig 5.5).

After onset of ischemia, both epicardial ($e_{max}$) and subendocardial maximal shortening ($e_{max,endo}$) decrease, the latter more pronounced in the first 10 s after occlusion. After one min of occlusion, however, the relative decrease in both variables is similar, which may be caused by a similar relation between $e_{max}$ and $e_{max,endo}$ as has been shown above to exist for $e_{min}$ and $e_{endo}$.

The relation between epicardial and subendocardial shortening in a particular direction suggests that shear between layers is indeed small enough to assess reliably the transmural course of deformation during ejection from epicardial deformation, as was already suggested to be possible during normoxia by Feigl and Fry (1964). A schematical representation of the relation between shortening in two myocardial layers, which fibers are directed perpendicular to each other, is

Fig 5.8
Proposed relationship between shortening in a direction in two different layers of the myocardial wall. Shortening along fiber A also causes shortening in the same direction of fiber B, located in a different layer and oriented perpendicular to fiber A.



Fig 5.9 Relation between the fiber direction at the epicardium and in the inner layers, and the direction of shortening in the various directions as derived from transmural deformation measurements. At the onset of ejection, two circles are assumed in both layers. Due to shortening during the ejection phase, the circles are deformed to ellipses. The form and orientation of the ellipses are conform the transmural deformation measurements. Shortening in all directions has been amplified by a factor 2.

shown in fig 5.8. Shortening in the fiber direction of one layer also
results in shortening in the same direction in other layers.

During normoxia, the model predicts fiber shortening in the inner
and outer layers to be the same (Arts, 1978). Measurements with ultra-
sonic crystals revealed larger shortening in the inner layers (Wein-
traub et al, 1981). In our experiments $e_{endo}$ was measured to be less
than half the value of $e_{max}$ at the epicardium. This might be due to
non-alignement of the orientation of $e_{endo}$ with respect to the fiber
direction in the inner layers. After all, the orientation of $e_{endo}$ is
defined to be perpendicular to the orientation of $e_{max}$. According to
the model prediction, $e_{max}$ is within 0.05 rad of the fiber direction
in the outer layers but the orientation of $e_{endo}$ is at an angle of 0.3
rad with the fiber direction in the inner layers (Arts et al, 1982).
This difference in orientation will cause $e_{endo}$ to be less than fiber
shortening in the inner layers. Nevertheless $e_{endo}$ provides informa-
tion on fiber shortening in these layers, as is shown in fig 5.9.

The present results show that during normoxia as well acute ische-
mia epicardial shortening along the direction of minimal shortening is
closely related to shortening in the inner layers along the same
direction, which is close to the fiber direction in these layers. This
is especially true if measurements can be referred to a control situa-
tion in the same animal (paired analysis) as is the case in the pre-
sent study.

## 5.4.2 Transmural differences in the changes of shortening along the approximate fiber direction

The early decline in shortening after acute coronary artery occlu-
sion is in agreement with findings of other investigators studying
regional ischemia (Banka and Helfant, 1974; Tyberg et al, 1974; The-
roux et al, 1976; Crozatier et al, 1977) or anoxia (Hearse, 1979).

The earlier decline in $e_{min}$ as compared with $e_{max}$ suggests that
after onset of coronary artery occlusion fiber shortening in the inner
layers is affected earlier than fiber shortening in the outer layers.
In chapter 6, it will be shown, that after onset of coronary artery
stenosis the time lag between the decrease of $e_{min}$ and $e_{max}$ is even
more pronounced.

The results from both deformation studies show that between 10 and

30 s after coronary artery occlusion lengthening occurs along a direction close to the fiber direction in the inner layers. After this time interval hardly any length changes occur during ejection. This disappearance of lengthening is only an apparent improvement, since it is caused by the earlier start of lengthening in the isovolumic phase. The decrease in lengthening may also be caused by increased myocardial stiffness (Edwards et al, 1981).

After one min of coronary artery occlusion, differences in the changes of shortening between the inner and outer layers were measured to be less than those found after 20 s. By studying shortening with ultrasonic crystals, Weintraub et al (1981) did not observe significant differences between the relative decreases in shortening in these two layers. Except for the measuring interval after flow reduction and the assessment of shortening during the whole systolic phase rather than the ejection phase, differences between their results and ours may arise from the orientation of the crystals. These authors positioned the subendocardial and subepicardial crystals parallel to the circumferential and base-to-apex axis, respectively. As can be seen from the data in fig 5.3, after one min of occlusion differences between circumferential and base-to-apex shortening were less than the differences between $e_{min}$ and $e_{max}$, suggesting that the relative decrease in fiber shortening might be slightly more pronounced in the inner than in the outer layers.

### 5.4.3 Comparison of measured and predicted transmural deformation

Previous experiments have been performed to verify the model (Arts et al, 1982). Values of epicardial deformation obtained in these experiments compared well with model calculations. Differences between the aforementioned experiments and those in the present study may originate from the differences in localization of the measuring device on the left ventricle between these two studies. The former has been performed near the base of the left ventricle whereas in this study deformation has been measured in the middle or apical region of this ventricle, because data had to be obtained from the center of the ischemic area. The basal region distinguishes itself from the middle and apical regions at various points, which may contribute to the observed difference between both studies.

Firstly, the presence of the anterior papillary muscle in the more apical region may influence the distribution and direction of shortening. Secondly, the apical geometry cannot be represented by a cylinder, which is the case in the basal region and is assumed in the model. At last, the fiber direction in the inner and outer layers is steeper in the apical than in basal region of the left ventricle (Streeter et al, 1969; Ross and Streeter, 1975). These differences in fiber orientation might influence regional transmural deformation. For the inner layers the model predicts the direction of minimal shortening to be at an angle of 1.4 rad with the circumference assuming a fiber direction of 0.75 rad in the inner subcylinder. In the apical region, fiber direction has been shown to be about 1.3 rad (Streeter et al, 1969). Because the minimal shortening direction at the epicardium will probably not be the same as the fiber direction in the inner layers and because relatively little fibers are circumferentially oriented in the apical region, the minimal shortening direction in the inner layers might be more circumferentially oriented in apical than in basal regions.

## 5.5 Conclusions

- Measurement of epicardial minimal shortening is closely related to shortening along the fibers in the inner layers.

- After onset of acute coronary artery occlusion shortening approximately parallel to the fiber direction in the inner layers ceases earlier (about 2 s) and more pronounced than shortening along the fiber direction in the outer layers.

- Within the first 30 s after onset of coronary artery occlusion large differences in fiber shortening between the inner and outer layers are found. After one min occlusion these differences are relatively small.

6. RELATIONSHIP BETWEEN FIBER SHORTENING, MYOCARDIAL BLOOD FLOW AND METABO-
LISM IN THE VARIOUS LAYERS OF THE LEFT VENTRICULAR WALL WITHIN 5 MINUTES
AFTER CORONARY ARTERY STENOSIS

6.1. Introduction

During ischemia myocardial blood flow (MBF) and metabolic processes are
known to be more severely affected in the inner than in the outer layers of
the left ventricle. Within a few seconds after onset of ischemia, myocardial
contraction is impaired (chapter 1). However, knowledge of the relation
between this performance, MBF and metabolic variables in the various layers,
is limited because information about the behavior of fiber shortening in the
various layers of the myocardial wall is difficult to obtain (section 2.1.3).
    In the previous chapter, measurement of maximal and minimal shortening of
the epicardial surface resulted in useful measures of shortening along the
epicardial fiber direction as well as shortening close to the fiber direction
in the inner layers.
    The purpose of the study described in this chapter was to investigate the
relation between the in this way measured shortening and MBF and metabolism
in the various layers of the myocardial wall as a function of time within the
first 5 min after coronary artery stenosis.

6.2 Experimental design

6.2.1 Protocol
    The experiments were performed on 30 mongrel dogs which were allotted to 3
groups according to the time of myocardial tissue sampling (e.g. 1, 3 and 5
min after onset of stenosis). To assure equal conditions before onset of
ischemia, care was taken, that between these 3 groups no significant diffe-
rences existed in heart rate, mean aortic pressure ($p_{ao}$) and $dp_{lv}/dt_{max}$.
Evaluation of the differences between the three aforementioned variables in
the three groups did not reveal any significant difference by applying the
Kruskal-Wallis test.
    The experimental protocol is shown in fig 6.1. For baseline measurements,
arterial and local venous samples were drawn 15, 5, and 0.5 min prior to

stenosis. The continuously recorded hemodynamic and regional mechanical variables were also determined at these moments. Cardiac output was determined 15 and 5 min prior to stenosis. Microsphere injection for the determination of regional myocardial blood flow was performed between 15 and 5 min prior to stenosis.

After these measurements had been performed, the occluder cuff was manually inflated until after 15-30 seconds post-stenotic coronary artery pressure had reached the desired value (i.e. 3.3 kPa; see section 3.1.4). Time 0 min was defined as the moment at which this pressure was reached. During stenosis, samples for determination of hemodynamic, blood biochemical and regional mechanical variables were taken 0.5, 1, 2, 3, 4 and 5 min after onset of stenosis. At each sample time the registration for the determination of the hemodynamic variables was made just before arterial blood sampling (fig 6.1). Local venous blood was continuously sampled from time 0 min till the end of the experiment. Blood withdrawn between times 0 and 0.5 min was called sample 0.5 min, between times 0.5 and 1 min was called sample 1 min and so on. Cardiac output was determined at about 0.75 min, 1.75 min, 2.75 min, 3.75 min and 4.75 min. Microspheres for the determination of regional myocardial blood flow were injected immediately after termination of arterial blood sampling at times 1, 3 and 5 min. Transmural biopsies for the determination of ATP, creatine phosphate and glycogen (section 3.1.6) from the ischemic (LAICA-) and normoxic (LCCA-) area were taken 15-30 s and 30-60 s after the last microsphere injection had been performed, respectively. After this procedure, the dogs were killed by an overdose of pentobarbital.

## 6.2.2 Data analysis

Information about the effect of ischemia was obtained by comparison of the data within each animal. The choice of each dog serving as its own control was made because of the large differences between animals. Influences on hemodynamical, regional mechanical and blood biochemical variables as well as MBF were evaluated by comparing the values at various time intervals after onset of ischemia with those at time -5 min. At each sample time changes in the content of the various substances within the ischemic myocardium (LAICA area) were evaluated by comparing the values of these contents with those in the non-ischemic tissue (LCCA area) in the same experiment. Indications for the correctness of this approach have been presented in chapter 4. The differences were evaluated for statistical significance by applying Wilcoxon's

Fig 6.1   Protocol of the experiments.



Fig 6.2   Time course of $e_c$, $e_z$, and $\gamma$ during 5 min of coronary   artery
stenosis. Median values and 95% limits are show
* $p<0.05$ as compared with time = -5 min.

matched-pairs signed-ranks test (two-tailed probability). The data are summarized as median values and 95% limits. A value of $p < 0.05$ was considered to be a significant difference.

## 6.3 Results

### 6.3.1. Inducement of stenosis

By manual inflation of the cuff, mean post-stenotic coronary artery pressure was reduced from 9.7 to 3.3 kPa (median values) within 10-30 s. Because of the use of the servosystem (section 3.1.4) this pressure hardly changed during the ischemic period and was approximately equal for all animals. (fig 6.3).

### 6.3.2 Time course of regional mechanical variables

The data obtained from the measurement of epicardial surface deformation are given in fig 6.2. During normal perfusion, values of circumferential shortening ($e_c$) were about twice the values of base-to-apex shortening ($e_z$). The median values 6.0% and 3.7% respectively. Analysis of the registration of these parameters in the first half min of stenosis in 11 animals revealed that shortening in both directions decreased already during the 10-30 s period of manual inflation of the cuff around the LAICA (not shown) and that $e_c$ transiently reached negative values, indicating the existence of circumferential lengthening during the ejection phase. After half a minute, shortening had decreased to median values of 0.1% ($e_c$) and 1.2% ($e_z$), indicating that the former variable is more sensitive to ischemia. From 30 s to 5 min after onset of stenosis, these values did not change significantly (fig 6.2).

In contrast to the shortening variables, the decrease in shear angle $\gamma$ was more gradual and less pronounced. In the first min after onset of stenosis $\gamma$ tended to increase transiently, but these changes did not reach the level of significance (inset in fig 6.2). After 5 min of stenosis the value of $\gamma$ was close to half the normoxic value (fig 6.2).

In chapter 5, it has been shown that minimal and maximal shortening ($e_{min}$ and $e_{max}$) as calculated from $e_c$, $e_z$ and $\gamma$ were useful markers of shortening in directions approximately parallel to the fiber direction in the inner and outer layers of the myocardial wall, respectively. The results of calculations based on the present data are shown in the figs 6.3 and 6.4. During inflation of the cuff, $e_{min}$ decreased already. The value of $e_{min}$ was negative

Fig 6.3 and 6.4    Time course of mean $p_{cor}$, $e_{min}$ and $e_{max}$ during the first 30 s (fig 6.3; n=11) and 5 min of acute coronary artery stenosis (fig 6.4; n=30). The first small arrow in fig 6.3 indicates the onset of decrease in mean $p_{cor}$, the second one indicates time 0 s, defined as the moment on which mean $p_{cor}$ reaches the appropriate level (= about 3.3 kPa). In fig 6.4 the arrow indicates the onset of stenosis. For explanation of symbols see fig 6.2.

when the cuff was appropriately inflated and decreased further until 20 s af-
ter onset of stenosis (fig 6.3). However, after one min, $e_{min}$ returned to
about zero and was stabile during the next 4 min (fig 6.4). In contrast to
the immediate decline in $e_{min}$, $e_{max}$ did not change significantly until 30 s
after onset of stenosis (fig 6.3). $e_{max}$ reached the lowest values (33%) of
the pre-ischemic values after one min of ischemia and remained constant
thereafter (fig 6.4).

## 6.3.3 Time course of regional myocardial blood flow

The MBF values as measured with the radioactive microsphere method are
shown in table 6.1. During normoxia MBF was almost homogeneously distributed
across the ventricular wall in the LAICA perfusion area. In the area perfused
by the LCCA, the perfusion of the outer layers was 10-25% lower than that of
the inner layers (discussed in detail in chapter 4). By inducement of steno-
sis of the LAICA, MBF in its perfusion area became inhomogeneous, and de-
creased much more in the inner layers than in the outer ones. From 1 to 5 min
after onset of stenosis, median MBF in the inner and outer layers further
decreased from 32% to 19% and from 64% to 46% of their pre-ischemic values,
respectively. The differences between the values at 1 and 5 min were not
statistically significant (table 6.1).

In the non-ischemic LCCA perfusion area, MBF significantly increased in
the outer but not in the inner layers. Therefore, the 10-15% increase in MBF
in the outer layers was associated with a small but significant decrease in
endo/epicardial flow ratio (table 6.1).

## 6.3.4 Time course of regional metabolic variables

The ATP, creatine phosphate and glycogen contents in the inner and outer
layers of the ischemic and non-ischemic area are shown in table 6.2. After 1,
3, and 5 min of ischemia, no significant decrease in ATP and glycogen content
was observed except for a decrease in the glycogen content of the outer
layers after 3 min of ischemia (table 6.2). In contrast, creatine phosphate
content rapidly decreased. Within 1 min of ischemia, creatine phosphate
content was halved in the inner layers of the myocardial wall, without signi-
ficant decrease in the middle (not shown) and outer layers (table 6.2). After
3 and 5 min of ischemia, creatine phosphate content was significantly decrea-
sed in all three layers; contents in the inner layers remaining significantly
lower than in the outer layers (table 6.2).

Table 6.1 Regional myocardial blood flow ($ml.min^{-1}.g^{-1}$) in the outer (EPI) and inner (ENDO) layers as well as the endo/epicardial blood flow (ENDO-EPI) ratio of the ischemic and non-ischemic tissue as well as the mean (transmural) blood flow of the ischemic tissue.
Median values and 95% limits are shown.

| Time (min) | -5 | 1 | 3 | 5 |
|---|---|---|---|---|
| **Ischemic region** | | | | |
| EPI | 0.83 | 0.53* | 0.44* | 0.38* |
| | 0.73-0.95 | 0.44-0.62 | 0.41-0.56 | 0.22-0.54 |
| ENDO | 0.76 | 0.24* | 0.23* | 0.15* |
| | 0.64-0.88 | 0.20-0.32 | 0.19-0.29 | 0.04-0.26 |
| ENDO-EPI RATIO | 0.96 | 0.50* | 0.51* | 0.45* |
| | 0.86-1.06 | 0.45-0.61 | 0.40-0.60 | 0.25-0.65 |
| Mean MBF | 0.78 | 0.40* | 0.34* | 0.28* |
| | 0.66-0.95 | 0.26-0.48 | 0.24-0.39 | 0.10-0.55 |
| **Non-ischemic region** | | | | |
| EPI | 0.7 | 0.86* | 0.83* | 0.86* |
| | 0.64-0.86 | 0.76-0.96 | 0.71-0.90 | 0.63-0.98 |
| ENDO | 0.85 | 0.90 | 0.90 | 0.78 |
| | 0.75-0.96 | 0.78-1.02 | 0.80-1.10 | 0.70-1.10 |
| ENDO-EPI RATIO | 1.17 | 1.11* | 1.14 | 1.07* |
| | 1.11-1.25 | 1.02-1.19 | 1.00-1.21 | 0.95-1.20 |

* $p < 0.05$ as compared to time -5 min.

Breakdown of energy-rich phosphates shortly after onset of stenosis was also indicated by the release of inorganic phosphate into the local venous blood which was significant within one min after this onset (table 6.3). Simultaneously with inorganic phosphate, potassium (table 6.3) and lactate (fig 6.5) were released into the blood. It can be seen that the release (calculated from the product of mean MBF in the ischemic area and the respective AV differences, section 3.7) of these substances rapidly increased during the first 3 min of ischemia, and hardly changed during the next 2 min.

The data depicted in fig 6.5 show a delay of 3-4 min between the changes in the AV differences of lactate and glucose. The median values of net glucose uptake revealed an initial decrease, that did not reach the level of significance. Therefore, glucose uptake returned to the pre-ischemic values due to the pronounced increase in AV differences (6-fold of median increase, value table 6.3).

AV differences of NEFA tended to increase in the first min after onset of stenosis (p=0.06) but returned to pre-ischemic values at the next two sample times. This resulted in a decrease of net NEFA uptake similar to the decrease in mean MBF in the ischemic area after 3 and 5 min (table 6.3). Oxygen uptake decreased from 3.8 at -5 min to 1.8 $\mu$mol.g$^{-1}$.min$^{-1}$ at -5 and 5 min (table 6.3).

## 6.3.5 Estimation of ATP utilization in the inner and outer layers during normoxia and ischemia

Myocardial energy metabolism was estimated from results in the present study and data published by other investigators. Oxygen uptake in the various layers was estimated from the measured oxygen uptake and the reported transmural gradient on oxygen uptake during normoxia and ischemia (Weiss et al, 1978; Weiss, 1980). From these values, aerobic ATP production in the inner and outer layers was calculated (table 6.4). Anaerobic ATP production was calculated to be 0.5 and 2.0 $\mu$mol.g$^{-1}$.min$^{-1}$ in the outer and inner layers, respectively, based on the reported lactate accumulation during ischemia (Griggs et al, 1972). The energy needed for basal metabolism and electrical processes has been reported to be 10-20% of total normoxic energy production (McKeever et al, 1958; Klocke, 1966). For the present calculation a value of 15% was assumed. According to these estimations, it follows that under normoxic conditions 16.7 and 19.4 $\mu$mol.min$^{-1}$.g$^{-1}$ ATP is used for mechanical processes in the outer and inner layers, respectively (table 6.4). After

Table 6.2 Contents of ATP, creatine phosphate and glycogen ($\mu$mol.g$^{-1}$ dry weight) before and during 5 min of ischemia. Median values and 95% limits are shown.

| | | Control | Ischemia | | |
|---|---|---|---|---|---|
| Time (min) | | | 1 | 3 | 5 |
| ATP | | | | | |
| epi | LAICA | 21 | 17 | 16 | 16 |
| | (ischemic) | 13-26 | 12-25 | 8-20 | 13-21 |
| | LCCA | 17 | 15 | 15 | 17 |
| | (non-ischemic) | 10-24 | 13-22 | 12-15 | 13-18 |
| endo | LAICA | 17 | 18 | 14 | 14 |
| | (ischemic) | 12-19 | 10-22 | 9-21 | 13-24 |
| | LCCA | 18 | 17 | 17 | 16 |
| | (non-ischemic) | 13-20 | 10-24 | 12-19 | 15-21 |
| Creatine phosphate | | | | | |
| epi | LAICA | 41 | 36 | 31* | 22* |
| | (ischemic) | 26-54 | 21-38 | 23-34 | 15-30 |
| | LCCA | 48 | 38 | 44 | 35 |
| | (non-ischemic) | 26-61 | 31-59 | 34-51 | 27-51 |
| endo | LAICA | 37 | 20*o | 23*o | 15*o |
| | (ischemic) | 29-53 | 14-26 | 12-46 | 11-20 |
| | LCCA | 39 | 51 | 39 | 40 |
| | (non-ischemic) | 25-51 | 35-60 | 30-56 | 24-76 |
| Glycogen | | | | | |
| epi | LAICA | 170 | 170 | 140* | 150 |
| | (ischemic) | 105-205 | 110-210 | 110-175 | 75-165 |
| | LCCA | 165 | 165 | 155 | 165 |
| | (non-ischemic) | 145-225 | 110-220 | 125-250 | 125-170 |
| endo | LAICA | 150 | 160 | 155 | 155 |
| | (ischemic) | 130-180 | 120-215 | 115-235 | 125-185 |
| | LCCA | 165 | 165 | 165 | 140 |
| | (non-ischemic) | 145-190 | 140-250 | 135-320 | 100-175 |

\* $p < 0.05$ as compared with the normoxic situation.
o $p < 0.05$ as compared with the outer layers.



Fig 6.5
Time course of the AV differences of lactate and glucose during 5 min of acute coronary artery occlusion (n=30). Remark that the scale for lactate is twice that for glucose. For explanation of the symbols see fig 6.2.

Table 6.3 Arterial concentrations (mM), AV differences (mM) and uptake ($\mu$mol.g$^{-1}$.min$^{-1}$) of substances in the blood before and during 5 min of ischemia. Medians and 95% limits are shown.

| Time (min) | -5 | 1 | 3 | 5 |
|---|---|---|---|---|
| **Oxygen** | | | | |
| Arterial | 7.3 | 7.3 | 7.4 | 7.0 |
| | 6.7-8.3 | 6.8-7.8 | 6.6-8.4 | 6.6-9.3 |
| AV diff. | 4.5 | 5.4* | 6.2* | 5.6* |
| | 3.9-5.2 | 4.9-5.9 | 5.0-7.1 | 4.5-7.9 |
| Uptake | 3.8 | 2.0* | 1.8* | 1.8* |
| | 2.9-4.2 | 1.3-2.4 | 1.3-3.1 | 0.3-2.9 |
| **Glucose** | | | | |
| Arterial | 6.4 | 6.2 | 6.2 | 6.7 |
| | 6.2-7.5 | 5.4-7.3 | 5.3-6.8 | 5.7-7.4 |
| AV diff. | 0.2 | 0.2 | 0.4* | 1.1* |
| | (-0.1)-0.5 | (-0.1)-0.5 | 0.2-0.9 | 0.3-2.3 |
| Uptake | 0.1 | 0.1 | 0.1 | 0.2 |
| | (-0.1)-0.5 | (-0.1)-0.2 | 0.0-0.3 | (-0.1)-0.8 |
| **Lactate** | | | | |
| Arterial | 1.86 | 1.93 | 1.67 | 1.73 |
| | 1.23-2.34 | 1.31-2.61 | 1.01-2.24 | 0.81-2.77 |
| AV diff. | 0.49 | -0.55* | -1.70* | -1.92* |
| | 0.30-0.61 | (-0.90)-(-0.30) | (-2.39)-(-1.10) | (-3.11)-(-0.50) |
| Uptake | 0.39 | -0.22* | -0.51* | -0.44* |
| | 0.21-0.65 | (-0.40)-(-0.06) | (-0.87)-(-0.27) | (-0.86)-(-0.40) |
| **NEFA** | | | | |
| Arterial | 0.31 | 0.28 | 0.28 | 0.24 |
| | 0.20-0.45 | 0.14-0.50 | 0.12-0.48 | 0.10-0.48 |
| AV diff. | 0.13 | 0.17 | 0.13 | 0.14 |
| | 0.09-0.17 | 0.06-0.27 | 0.05-0.28 | 0.01-0.31 |
| Uptake | 0.10 | 0.07* | 0.04* | 0.04* |
| | 0.06-0.14 | 0.03-0.08 | 0.02-0.07 | 0.00-0.09 |
| **Inorganic phosphate** | | | | |
| Arterial | 1.70 | 1.72 | 1.71 | 2.08 |
| | 1.35-1.84 | 1.53-1.94 | 1.46-2.02 | 1.62-2.21 |
| AV diff. | -0.02 | -0.30* | -0.70* | -0.90* |
| | (-0.08)-0.00 | (-0.43)-(-0.22) | (-0.88)-(-0.52) | (-2.35)-(-0.56) |
| Uptake | -0.02 | -0.08* | -0.23* | -0.26* |
| | (-0.08)-(-0.02) | (-0.11)-(-0.05) | (-0.33)-(-0.10) | (-0.68)-(-0.05) |
| **Potassium** | | | | |
| Arterial | 3.55 | 3.75 | 3.73 | 3.65 |
| | 3.35-3.90 | 3.35-4.00 | 3.40-4.05 | 3.55-4.45 |
| AV diff. | -0.04 | -0.75* | -1.15* | -1.10* |
| | (-0.14)-0.02 | (-1.00)-(-0.60) | (-2.05)-(-0.95) | (-2.45)-(-0.75) |
| Uptake | -0.04 | -0.23* | -0.34* | -0.33* |
| | (-0.15)-0.07 | (-0.35)-(-0.17) | (-0.72)-(-0.18) | (-1.02)-(-0.09) |

* p<0.05 as compared with time -5 min

one min of ischemia, assuming energy for basal metabolism and electrical activity to be constant, only 14.4 and 9.3 $\mu$mol.g$^{-1}$.min$^{-1}$ ATP is available for mechanical activity in the outer and inner layers, respectively (table 6.4).

### 6.3.6 Time course of hemodynamic variables

In table 6.4, the values of several hemodynamic variables are shown before and during 5 min of coronary artery stenosis. Heart rate gradually increased by 11% and mean aortic pressure gradually decreased by 12% during 5 min of ischemia. Within 1 min of ischemia, $dp_{lv}$/dtmax decreased by 23% and $p_{lved}$ increased by 25% (p<0.05). These changes became more pronounced during the next 4 min of ischemia. Median values of cardiac output decreased, but this decrease was not significant.

These results show that the inducement of ischemia resulted in a decreased global left ventricular function, but without a significant decrease in cardiac output and the occurrence of serious arrhythmias.

### 6.4 Discussion

### 6.4.1 Effects of ischemia on general hemodynamics

By stenosis of the LAICA, 10 to 20% of the left ventricle is rendered ischemic (section 3.4). Impaired mechanical function in this area exerts significant influences on the hemodynamic variables. The loss of contrac- tility of the ventricle as a whole is reflected by the decreased $dp_{lv}$/dtmax, while cardiac output tended to decrease. This loss in contractility hampers the ejection of blood as is also shown by the increase in $p_{lved}$. The increase in heart rate is probably reflexogenic and secondary to the decrease in aortic pressure.

Despite these significant effects on global hemodynamics, cardiac output was hardly affected. This might be partly due to compensatory mechanical function of the non-ischemic area as suggested by the increased MBF in this area. In the early phase of ischemia, slight but non significant increases in non-ischemic MBF were also observed by Driscoll and Eckstein (1964), Domenech (1974) and Rivas et al (1976).

### 6.4.2 Effects of ischemia on myocardial blood flow

Stenosis of the coronary artery leads to a fall in post-stenotic coronary

artery pressure and coronary flow. The degree of stenosis used in the present study caused a significant 50% fall in mean MBF in the ischemic area after one min of stenosis, slightly but not significantly decreasing in the next 4 min.

The inhomogeneous distribution of MBF across the ischemic left ventricular wall, resulting in a relative underperfusion of the inner layers, has been extensively described (chapter 1). This is caused by the higher intramyocardial pressure in the inner than in the outer layers (Hoffman, 1979; Arts et al, 1979).

## 6.4.3 Effects of ischemia on myocardial metabolism

The reduction of myocardial blood flow results in an impaired oxygen delivery to the affected perfusion area. The oxygen shortage causes rapid and pronounced changes in metabolism of the ischemic area, as indicated by the release of lactate, inorganic phosphate and potassium within 1 min after onset of stenosis. These observations are in agreement with the findings of Obeid et al (1972) and Opie et al (1973) as well as with the results of Gudbjarnason et al (1970) who showed tissue lactate accumulation within 30 s after coronary artery occlusion. The net lactate release during the first 5 min of ischemia exceeded the net glucose uptake in that period. This observation suggests anaerobic breakdown of other carbohydrate sources (e.g. glycogen) during this early phase of ischemia. Median values of glycogen content were lower in the ischemic than in the non-ischemic tissue but the difference was only significant in the outer layers after 3 min of ischemia. However, a decrease of 1 $\mu$mol.g$^{-1}$ glycogen(glucose), which is within the interindividual variation, easily covers this additional lactate release. Such a rapid enhancement of glycogenolysis cannot be excluded, since at the beginning of ischemia the inactive phosphorylase b is rapidly converted into the active phosphorylase a (Kuebler and Spieckermann, 1970; Ichihara and Abiko, 1977) and phosphorylase b itself is activated within one min of ischemia (Wollenberger and Krause, 1968). Moreover, after inducement of anoxia in an isolated heart preparation, lactate release was not severely depressed if a glucose-free perfusion was employed (Opie, 1971/72).

The delayed change in glucose AV differences, as compared to those for lactate, is incompletely understood. A relatively slow activation of the carrier mediated glucose uptake into the cells is one possibility. A rapid increase of glucose uptake simultaneously with a release of glucose units

through early glycogenolysis (see above) is not likely to occur, because heart muscle does not contain glucose-6-phosphatase (Stetten, 1964).

The observed potassium release could indicate changes in the function of the sodium-potassium pump or the potassium conductance of the sarcolemma. Rau and Langer (1978) observed that potassium loss during anoxia continues, despite, uncoupling of excitation and contraction or increasing glycolytic energy production. However, no potassium loss occurred when the preparation was electrically quiescent. Because the sodium-potassium pump continued to function until 60 min of anoxia (Rau et al, 1977), it was concluded that the potassium loss is mainly caused by a higher potassium conductance. Involvement of a membrane oxygen sensing system (Meduski et al, 1977) has been proposed.

Increased potassium conductance has been related to the origin of arrhythmias (Coraboeuff et al, 1976). However, severe arrhythmias did not occur when ischemia was induced in our preparation as well as in that of Owen et al (1970) and of Opie et al (1973).

## 6.4.4 Relation between cessation of shortening and myocardial blood flow and metabolism

Due to oxygen shortage, energy supply for contractile activity will be impaired. After inducement of ischemia as well as anoxia, decreasing contractile activity did not coincide with depletion of energy-rich phosphate stores in the whole myocardial wall or in samples from the outer layers (Braasch et al, 1968; Gudbjarnason et al, 1970; Hearse, 1979; Imai et al, 1979). The present study shows that in the ischemic area cessation of fiber shortening in the inner layers occurs without depletion of energy-rich phosphate stores in these layers.

The absence of a tight relation between ATP content and contractile function is even better illustrated by the finding that during catecholamine-induced stimulation, ATP values decrease to values lower than those during acute ischemia, but without impairment of contractile function (Kammermeier et al, 1974). Moreover, it is well known that during reperfusion after complete ischemia, mechanical recovery occurs while ATP contents remain depressed (Hearse, 1974).

To explain cessation of contractile activity despite the presence of energy-rich phosphates, several alternative hypotheses have been proposed, which are summarized in section 2.1.2, and will be discussed here in relation to

the results obtained in the present study.

Accumulation of NEFA as a possible cause of early mechanical failure as proposed by Katz and Messineo (1981), is not likely. Uptake of NEFA from the blood ($0.07\ \mu$mol. $g^{-1}.min^{-1}$) may cause an increase in cellular NEFA concentration of 70 nmol.$g^{-1}$. Since still 2 $\mu$mol.$g^{-1}$ oxygen is taken up and the uptake of glucose has decreased, most of these NEFA will be oxidized. The remaining NEFA might well be coupled to free CoA or carnitine. Therefore, it seems likely that cellular NEFA concentrations are hardly affected within 1 min of ischemia. Moreover, in the next chapter it will be shown that the content of these substances does not increase before 10 min of ischemia.

Glycogen depletion has been reported to occur within 4 min of anoxia in isolated hearts. Simultaneously, ATP synthesis and mechanical activity were impaired (Rovetto, 1979). The findings in the present study, however, show that no depletion of glycogen occurs within 5 min after onset of ischemia and hence glycogen depletion is not likely to be the cause of the early cessation of fiber shortening. These contradictory results may be explained by a higher glycolytic flux during anoxia (Liedtke, 1981).

The rapid decrease of pH in local venous blood after onset of stenosis indicates an even more pronounced decrease in cellular pH. A proton induced change in calcium homeostasis has been suggested as a possible trigger for contractile failure. This would be induced by decreased affinity of troponin for calcium at high hydrogen ion concentrations (Katz and Hecht, 1969) or by interference of protons with calcium movements during the action potential (reviewed by Nayler et al, 1979). As mentioned in section 2.1.2, an important role of pH in the deterioration of mechanical function is rather unlikely. This is supported by the observation that the decline in pressure development in the anoxic isolated working rat heart preparation (Hearse, 1979) occurs at a similar time interval as the cessation of shortening after acute coronary artery occlusion (chapter 5), despite the maintained flow and washout of carbon dioxide and protons during anoxia.

Whereas the above mentioned theories bypass the involvement of energy-rich phosphate breakdown in early contractile failure, several recent theories stress the major role of this process in the inhibition of contraction.

Breakdown of energy-rich phosphates after oxygen deprivation will result in increased cellular concentrations of inorganic phosphate. High cellular inorganic phosphate concentrations were considered to be responsible for the binding of considerable amounts of calcium (Kuebler and Katz, 1977). These

authors calculated an 8 fold increase in this substance during ischemia. Indeed,the early inorganic phosphate release after onset of stenosis, likely reflects elevated cellular concentrations of this substance. However, as can be calculated from results of the present study the amount of inorganic phosphate liberated from energy-rich phosphates within one min of ischemia is no more than 4 $\mu$mol.g$^{-1}$ wet weight in the inner layers of the myocardium, which increase would be less than two-fold the normoxic tissue content. Because of the release of this substance into the blood, the actual concentration will even be lower. These findings are comparable to the values found by Imai et al (1979) in the isolated guinea pig heart after 0.5 and 1 min of ischemia and are far below the increase in inorganic phosphate concentration as estimated by Kuebler and Katz (1977; section 2.1.2).

The observed negative AV differences of inorganic phosphate (already after 0.5 min of stenosis significantly different from the pre-ischemic value) indicate rapid breakdown of energy-rich phosphates, most likely creatine phosphate. This is consonant with an early decrease in the phosphorylation potential, experimentally shown to occur in the ischemic guinea pig heart within 0.5 min. This decrease was synchronous with the decrease in mechanical performance (Imai et al, 1979). Similar findings were reported by Kammermeier et al (1981) in the hypoxic isolated rat heart. Moreover, these authors calculated a decreased phosphorylation potential from estimated cytoplasmatic concentrations instead of gross tissue contents. An interesting consequence of this theory is that breakdown of creatine phosphate could directly impair mechanical function, even in the presence of sufficient ATP.

Approval nor disapproval is obtained in the present study on the pressence of different cellular compartments for ATP as proposed by Gudbjarnason et al (1970; section 2.1.2).

## 6.4.5 Changes of mechanical activity in various layers of the left ventricular wall during ischemia

Fiber shortening decreases more rapidly in the inner than in the outer layers, as indicated by the earlier decline in $e_{min}$ as compared to $e_{max}$. This phenomenon is likely to be related to the more pronounced decrease of blood flow in the inner layers (68%), than in the outer layers within one min of coronary artery stenosis. Consistent with this finding is, that after acute coronary artery occlusion $p_{O2}$ in the inner layers decreased at a higher rate and to lower values as compared to the outer layers (Winbury et al, 1979).

The higher energy demand in the inner layers than in the outer ones, as indicated by the higher oxygen uptake in the former layers (Holtz et al,1977; Weiss et al, 1978), might also play a role in the earlier diminishing of fiber shortening in the former layers. The disappearance of lengthening along the fiber direction of the inner layers during ejection after one min of ischemia is probably no improvement of mechanical performance since it is associated with an early increase in segment length at the onset of ejection. This lengthening before ejection, occurring during ischemia, was also found by Edwards et al (1981). Also the almost immediately increasing stiffness of the ischemic tissue (Theroux et al, 1974, 1977; Edwards et al, 1981) may reduce lengthening during ejection along the fiber direction in the inner layers.

During the interval between the decrease of $e_{min}$ and $e_{max}$, considerable differences in shortening along the fiber direction are present between inner and outer layers of the left ventricle. One min after onset of ischemia, however, transmural differences in shortening have become relatively small. Downey (1976) studied the contractile force in the inner and outer layers, using epicardially mounted strain gauges oriented parallel to the fiber direction of the inner and outer layers, a set-up which is comparable to the one used in this study. He also observed relatively small differences between "superficial and deep force". The results from their study and ours provide qualitative indications and thus do not allow conclusions about absolute differences in fiber shortening in the various layers. Using ultrasonic crystals, Weintraub et al (1981) recently showed that during various degrees of ischemia the relative decrease in shortening in the inner and outer layers was not significantly different.

The relatively small differences in the changes of shortening are in contrast with the steep gradients in myocardial blood flow and creatine phosphate contents between the various myocardial layers. After one min of ischemia in the outer layers blood flow had decreased by only 36% and crea- tine phosphate content was not significantly affected in contrast to a 64% decrease in blood flow and halved creatine phosphate stores in the inner layers. A possible explanation for this paradoxal situation might be a redis- tribution of fiber stress across the wall. The outer layers may have to bear more stress due to failure of the inner layers. Several arguments may support this hypothesis.

The slower decrease of $e_{max}$ compared to $e_{min}$, shown in this chapter and in

chapter 5, suggests that after diminishment of shortening in the inner layers, the fibers in the outer layers still try to shorten. After acute coronary artery occlusion (chapter 5), fibers in the outer layers continue to shorten for only several seconds, whereas after stenosis (fig 6.3) this shortening continues for half a minute. This difference may be explained by the higher blood supply to the ischemic area than in the latter situation, enabling the outer layers to persist mechanical function for a longer time.

Recent studies showed that in the inner layers the decrease in shortening is related to the decrease in blood flow, but that such a relationship does not exist in the outer layers. However, the decrease in fiber shortening in the outer layers proved to be related to the decrease in fiber shortening in the inner layers, suggesting that subendocardial ischemia results in a transmural redistribution of mechanical loading and contractile function (Gallagher et al, 1978; Genain et al, 1979; Weintraub et al, 1981).

The bearing of compensatory stress by the outer layers is also suggested by estimated ATP utilization in the inner and outer layers (table 6.4). Because mechanical activity in the ventricle depends mostly on stress development of the fibers, and little differences seems to be present between shortening in the inner and outer layers, the difference in estimated energy utilization between these layers suggests, that the contractile mechanism in the inner layers develops less stress due to the pronounced energy shortage. The increased workload thus caused in the outer layers may result in a decrease in fiber shortening in these layers to such an extent that the transmural differences in fiber shortening are not as large as the transmural differences in blood flow or creatine phosphate contents.

## 6.5 Conclusions

- The first measurable effect after inducement of ischemia is a decrease in regional fiber shortening, which occurs within half a minute. Within this short time interval large differences in fiber shortening are present between the various layers, since fiber shortening cessates earlier and more pronounced in the inner layers than in the outer ones.
- Cessation of regional shortening occurs without a decrease in ATP content and with a decrease in creatine phosphate content only in the inner layers. these results indicate, that depletion of energy-rich phophates is not the cause of diminishing local pump function after onset of ischemia.

- Of the causes of this early contractile failure mentioned in the literature (section 2.1.2), decrease of the energy provided by hydrolysis of one mol ATP most adequately explains the results from the present study.
- During ischemia of longer duration than one min, transmural differences in fiber shortening are relatively small despite the higher oxygen delivery in the outer layers than in the inner ones. This observation suggests that during ischemia, the outer layers are impeded to shorten by the inner layers.
- Early lactate release cannot completely be explained by the net uptake of glucose, which might be explained by early glycogen breakdown.
 - The rapidly declined mechanical activity in the ischemic area exerts significant effects on general hemodynamic variables. Increased MBF in the non-ischemic area suggests increased mechanical activity in this area to compensate for function loss by the ischemic tissue.

# 7. TIME COURSE OF CHANGES IN BLOOD FLOW, LOCAL MECHANICS AND METABOLISM IN MYOCARDIAL TISSUE DURING 120 MIN OF ISCHEMIA WITH SPECIAL REFERENCE TO ACCUMULATION OF NEFA

## 7.1 Introduction

The content of non-esterified fatty acids (NEFA) in ischemic myocardium was reported to be increased (Weishaar et al, 1977, 1979; Van der Vusse et al, 1982). Several studies showed that high intracellular or extracellular NEFA concentrations exerted deleterious effects on the activity of several enzymes and on membrane function (reviewed by Katz and Messineo, 1981; Van der Vusse, 1982). If NEFA, accumulated during ischemia, have noxious effects on the oxygen-restricted area, one would expect that this accumulation pre-ceeds the deterioration of the processes proposed to be affected in this region.

Therefore, it is interesting to compare the time course of this accumu-lation with that of the content of other lipid classes, glycogen and high energy phosphates as well as the time course of blood flow and fiber shor-tening in the ischemic myocardium.

Changes in the afore-mentioned variables were determined in the outer, middle and inner layers of the free wall of the left ventricle since reduc-tion of coronary artery blood flow results in marked underperfusion of the inner layers, which might cause transmural differences in myocardial meta-bolism of the afore-mentioned substances as well as in mechanical performance of these layers.

An indication about the contribution of extracellular and intracellular sources to NEFA accumulation was made possible by gaschromatographic determi-nation of the content of the individual fatty acids in the NEFA, triacylgly-cerol and phosphoglyceride classes in the myocardium as well as the arterio-local venous (AV) differences of the individual fatty acids of the two first mentioned classes (section 3.7).

## 7.2 Experimental design

### 7.2.1 Protocol
The experiments were performed on 30 mongrel dogs which were allotted to 3

groups, according to the time of myocardial tissue sampling (e.g. 10, 60 and 120 min after onset of ischemia). To assure similar conditions before onset of ischemia, care was taken that between these 3 groups, no differences existed in heart rate, mean aortic pressure ($p_{ao}$) and $dp_{lv}/dt$ max. Moreover, to obtain a comparable degree of stenosis in the animals of the three relatively small groups, allotting an animal to a certain group occurred so, that after 10 min of ischemia mean $p_{cor}$ and the change in pH and $O_2$ saturation in the local venous blood were comparable between the groups. These variables were chosen to assess the degree of ischemia because (a) they can be determined during the experiment, (b) a decrease in local venous pH reflects lactate production by the ischemic myocardium, (c) similar values of mean $p_{ao}$ and $p_{cor}$ assure a comparable degree of stenosis in the groups. Evaluation of the differences between the 6 afore-mentioned variables in the 3 groups did not reveal any significant difference by applying the Kruskal-Wallis test indicating that the procedure of allocation had been successful.

The protocol of these experiments is shown in fig 7.1. Stenosis was induced at time 0 min by inflation of the occluder cuff until the desired $p_{cor}$ was reached (3.3 kPa). In these experiments, arterial and local venous blood samples were taken at times -15, -5, 10, 20, 30, 45, 60, 90, and 120 min. The continuously recorded hemodynamic and regional myocardial mechanical variables as well as cardiac output were also determined at these moments. Radioactive microspheres for determination of regional myocardial blood flow were injected just after times -15, 10, 60, and 120 min. Transmural biopsies were taken from the ischemic (LAICA) and non-ischemic (LCCA) area just after the microsphere injections, 10, 60, or 120 min after onset of stenosis, depending on the group in which they were performed. In all experiments, biopsies were taken for the determination of ATP, creatine phosphate and glycogen. In 5 experiments of each group, also biopsies were taken for the determination of fatty acids (section 3.1.6). In these 15 experiments the biopsies for the determination of ATP, creatine phosphate and glycogen were taken just prior to those for fatty acid determination. Four additional experiments were performed, including microsphere injections and biopsies for fatty acid determination after 60 min of ischemia. At the end of the experiments, the dogs were killed by an overdose of pentobarbital.

For the analysis of the NEFA contents in myocardial tissue and the relation between these contents and myocardial blood flow, we also used the data from biopsies, taken in separate experiments after 120 min of normal perfu-

Fig 7.1 Protocol of the experiments.

Table 7.1 Time course of several hemodynamic variables before and during 120 min of ischemia. Median values and 95% limits are shown.

| Time (min) | -5 | 10 | 60 | 120 |
|---|---|---|---|---|
| Heart rate ($b.min^{-1}$) | 116 85-160 | 130* 95-175 | 124 85-155 | 113 65-150 |
| Mean aortic pressure (kPa) | 10.8 10.3-11.3 | 10.5* 9.9-11.1 | 10.8 9.7-11.5 | 9.5* ** 8.7-10.1 |
| Mean coronary artery pressure (kPa) | 10.5 9.9-11.1 | 3.3* 3.2-3.5 | 3.2* 2.9-3.5 | 3.2* 2.8-3.6 |
| $p_{lved}$ (kPa) | 0.6 0.4-0.8 | 0.8* 0.7-1.1 | 1.2* 0.7-1.5 | 0.7* 0.4-1.3 |
| $dp_{lv}/dtmax$ ($kPa.s^{-1}$) | 206 173-240 | 181* 147-220 | 160* 120-190 | 133* 93-173 |
| Cardiac output ($l.min^{-1}$) | 2.9 2.4-3.4 | 2.6* 2.2-3.2 | 2.5* 2.2-2.7 | 2.4* 1.9-2.9 |
| Stroke volume (ml) | 28 22-33 | 23* 17-27 | 21* 16-24 | 20* 17-30 |

* p<0.05 as compared with t= -5 min
** p<0.05 as compared with t= 60 min

sion (control experiments, n=5, 10 biopsies from the LAICA area and 10 biop-
sies from the LCCA) and after 120 min of stenosis (n=8, 16 biopsies from the
ischemic LAICA area and 16 biopsies from the normoxic LCCA area). These data
have been published recently (Van der Vusse et al, 1982).

## 7.2.2 Data analysis

Information about the effect of ischemia was obtained by comparison of the
data within each animal as well as between groups of animals.

The choice of each dog serving as its own control was made because of the
great differences between animals. Influences on hemodynamical, regional
mechanical and blood biochemical variables as well as MBF were evaluated by
comparing the values at various intervals after onset of ischemia with those
at time -5 min. At each sample time, changes in the content of the various
substances within the ischemic myocardium were evaluated by comparing the
values of these contents with those of the non-ischemic tissue in the same
experiment (validated in chapter 4). These differences were evaluated for
statistical significance by applying Wilcoxon's matched-pairs signed-rank
test (two-tailed probability).

Differences in tissue contents of the various substances in the LAICA and
LCCA area between groups of experiments without stenosis (for fatty acids:
data in Van der Vusse et al, 1982; for ATP, creatine phophate and glycogen:
data presented in chapter 4) and including the inducement of stenosis, were
evaluated for statistical significance by applying the two-tailed Wilcoxon's
rank-sum test (aligned ranks).

The data are summarized as median values and 95% limits. A value of $p<0.05$
was considered to be a significant difference.

## 7.3 Results

### 7.3.1 Time course of hemodynamic variables during 2 hours of regional ische-
    mia

The effect of acute coronary artery stenosis on these variables is shown
in table 7.1. Using the servo system, post-stenotic coronary artery pressure
was kept constant at about 3.3 kPa with less than 0.3 kPa intra-individual
variation throughout the experimental period and the same variation between
animals.

After onset of stenosis, heart rate increased within 1 min (section 6.3.6)

Table 7.2 Regional myocardial blood flow ($ml.min^{-1}.g^{-1}$) in the outer (EPI), middle (MID), and inner (ENDO) layers of the left ventricle, the endo/epicardial blood flow (ENDO/EPI) ratio of the ischemic and non-ischemic tissue as well as the mean transmural blood flow of the ischemic tissue before and during 120 min of ischemia. Median values and 95% limits are shown.

| Time (min) | -5 | 10 | 60 | 120 |
|---|---|---|---|---|
| **Ischemic region** | | | | |
| EPI | 0.83 | 0.30* | 0.34* | 0.35* |
| | 0.73-0.95 | 0.25-0.41 | 0.22-0.41 | 0.18-0.59 |
| MID | 0.73 | 0.18* | 0.19* | 0.19* |
| | 0.64-0.85 | 0.16-0.24 | 0.13-0.24 | 0.09-0.30 |
| ENDO | 0.76 | 0.15* | 0.13* | 0.10* ** |
| | 0.64-0.88 | 0.12-0.22 | 0.07-0.19 | 0.04-0.18 |
| ENDO/EPI RATIO | 0.96 | 0.50* | 0.34* ** | 0.28* ** |
| | 0.86-1.06 | 0.40-0.60 | 0.28-0.42 | 0.15-0.40 |
| Mean MBF | 0.74 | 0.24* | 0.24* | 0.17* |
| | 0.52-0.98 | 0.13-0.30 | 0.12-0.35 | 0.11-0.34 |
| | | | | |
| **Non-ischemic region** | | | | |
| EPI | 0.75 | 0.82* | 0.71 | 0.63 |
| | 0.64-0.86 | 0.68-1.00 | 0.60-0.82 | 0.55-0.98 |
| MID | 0.80 | 0.88* | 0.75 | 0.68 |
| | 0.68-0.92 | 0.75-1.03 | 0.65-0.84 | 0.48-0.91 |
| ENDO | 0.85 | 0.87 | 0.75 | 0.68 |
| | 0.75-0.96 | 0.76-0.98 | 0.62-0.89 | 0.49-0.89 |
| ENDO/EPI RATIO | 1.17 | 1.15 | 1.15 | 1.13 |
| | 1.11-1.25 | 1.05-1.26 | 1.04-1.23 | 0.93-1.36 |

\* $p < 0.05$ as compared with time -5 min
\** $p < 0.05$ as compared with time 10 min



Fig 7.2
NEFA content in tissue samples from the LAICA perfusion area of animals without inducement of stenosis ("control") and from animals after 10, 60, or 120 min of ischemia. Shown are the median values and 95% limits of the outer (epi), middle (mid), and inner layers (endo). * $p < 0.05$ as compared with the control group.

and remained constant for 10 min. Thereafter heart rate gradually decreased, reaching preischemic values after 120 min. Mean aortic pressure slightly but significantly decreased simultaneously with the increase in heart rate, and returned to pre-ischemic values after 60 min. During the second hour of ischemia, the median values of mean aortic pressure decreased by 12% which was statistically significant. The maximal positive first derivative of left ventricular pressure ($dp_{lv}/dtmax$) continued to decrease after the initial fall during the first 5 min of ischemia (section 6.3.6). After 120 min of ischemia, the values of this variable had decreased to 65% of the preischemic values. End-diastolic left ventricular pressure steadily and significantly increased to 133 and 200% of the pre-ischemic values at 10 and 60 min of ischemia, respectively, but thereafter tended to return to the preischemic values although no significant difference could be detected between the values at time 60 and 120 min ($p=0.11$). The median values of cardiac output during ischemia were approximately 15% below the pre-ischemic values. Due to the slightly higher heart rates during ischemia, the fall in stroke volume was more pronounced (30% after 120 min).

### 7.3.2 Time course of regional myocardial blood flow

The inducement of ischemia caused an inhomogeneous distribution of MBF. In the outer layers MBF decreased by about 60% after 10 to 120 min. After 10 min of ischemia, MBF in the inner layers was already reduced by about 80% and continued to decrease to a reduction of about 87% after 120 min. The transmural gradient in MBF between the inner and outer layers is expressed in the endo/epicardial blood flow ratio. This ratio decreased from 0.96 at time -15 min to 0.50 after 10 min and 0.28 after 120 min of ischemia (table 7.2).

After 10 min of stenosis, in the non-ischemic area blood flow significantly increased in the outer (9%) and middle layers (10%), but the increase of 2% in the inner layers did not reach the level of significance (table 7.2). After ischemia of longer duration, blood flow in this area decreased so that the values at time 120 min tended to be lower than those at time -15 min. In the normoxic area, the transmural distribution of myocardial blood flow did not change during the whole experimental period (table 7.2).

### 7.3.3 Time course of myocardial fatty acid contents during ischemia

After 10 min of ischemia, NEFA contents in the three studied layers of the ischemic area were not significantly different from those in the three layers

Fig 7.3 Content of the individual NEFA in myo-
cardial tissue samples from the outer
(7.3a) and inner (7.3b) layers of the
myocardium in the groups mentioned in
fig 7.2. Median values and 95% limits
are shown.

Table 7.3
Ratio of ischemic con-
tent and non-ischemic
content of the indivi-
dual fatty acids in the
inner and outer layers
of the ischemic myocar-
dium, as calculated
from the respective me-
dian values after 10,
60 and 120 min of is-
chemia.



outer layers

NEFA ($nmol.g^{-1}$)

| Time (min) | 10 | 60 | 120 |
|---|---|---|---|
| Outer layers | | | |
| 14:0 | 1.5 | 1.9 | 1.8 |
| 16:0 | 1.0 | 2.3 | 1.6 |
| 16:1 | 0.7 | 2.3 | 1.6 |
| 18:0 | 0.9 | 3.2 | 1.5 |
| 18:1 | 1.1 | 4.3 | 2.3 |
| 18:2 | 0.9 | 5.6 | 2.5 |
| 20:4 | 1.0 | 3.4 | 1.7 |



inner layers

NEFA ($nmol.g^{-1}$)

| Inner layers | | | |
|---|---|---|---|
| 14:0 | 1.3 | 1.3 | 2.5 |
| 16:0 | 1.1 | 1.8 | 3.4 |
| 16:1 | 0.7 | 2.0 | 3.6 |
| 18:0 | 1.2 | 2.9 | 3.8 |
| 18:1 | 1.8 | 2.5 | 5.8 |
| 18:2 | 1.4 | 3.5 | 7.3 |
| 20:4 | 1.1 | 4.2 | 8.1 |

of the non-ischemic LAICA area in the control experiments. However, after 60 and 120 min, these substances accumulated in the ischemic area (fig 7.2). In the outer layers, NEFA contents increased more than 3-fold after 60 min but only 1.6-fold after 120 min as compared with the aforementioned control values. In contrast to this biphasic time course in the outer layers, the NEFA content in the inner layers increased approximately 2-fold after 60 min and 4-fold after 120 min of ischemia. The middle layers of the ischemic myocardium did not show an increased NEFA content after 60 min, but one hour later the NEFA content in these layers was intermediary between the content in the inner and outer layers (fig 7.2).

The individual NEFA content in tissue samples from the normoxic LAICA area in the control experiments and in tissue samples after 10, 60 and 120 min of ischemia, both in the outer and inner layers are shown in fig 7.3. In general, an increase in total NEFA content was caused by higher contents of each individual fatty acid. However, differences existed in the relative increase of these fatty acids (table 7.3, fig 7.3). At the maximum NEFA accumulation in the outer layers (after 60 min) relative increases in linoleic and oleic acid were most predominant. The content of all individual NEFA in the inner layers was significantly increased after 60 min of ischemia and gradually increased thereafter. The fatty acids with the highest relative increase after 120 of ischemia were arachidonic and linoleic acid (8.1 and 7.3-fold increase, respectively; fig 7.3, table 7.3). Despite the unchanged total amount of NEFA in the middle layers after 60 min of ischemia, small but significant increases were observed in the arachidonic and linoleic acid content, (1.7 and 1.6-fold increase, respectively). These fatty acids also showed the highest relative accumulation (4-fold, both) in this layer after 120 min of ischemia (data not shown).

Intra-individual comparison of the content of triacylglycerol in the ischemic and non-ischemic area showed lower values in the non-ischemic area (significant after 120 min in the middle and after 60 and 120 min in the inner layers; table 7.4). This difference was due to a decreased content of triacylglycerol in the non-ischemic (LCCA) area as was shown by comparing these contents with those from the LCCA area in experiments without inducement of stenosis (data in Van der Vusse et al, 1982). The triacylglycerol content in the ischemic and normoxic LAICA (control group) area were not significantly different (table 7.4).

The values of the phosphoglyceride contents in the ischemic and non-ische-

Table 7.4 Contents of triacyglycerol and phosphoglyceride ($\mu$mol.g$^{-1}$) in animals without (control, n=10) and with stenosis of the LAICA during 10 (n=10), 60 (n=18), and 120 min (n=30). Median values and 95% limits are shown.

| | | Control | Ischemia | | |
|---|---|---|---|---|---|
| Time (min) | | | 10 | 60 | 120 |
| Triacylglycerol | | | | | |
| epi | LAICA | 14.1 | 6.7 | 10.2 | 7.5 |
| | (ischemic) | 5.7-21.1 | 4.6-12.2 | 8.4-12.6 | 5.4-13.7 |
| | LCCA | 11.0 | 8.2 | 11.2 | 10.0 |
| | (non-ischemic) | 6.4-35.0 | 3.3-31.7 | 7.7-15.3 | 3.0-16.3 |
| endo | LAICA | 5.7 | 9.0 | 8.6 | 5.7 |
| | (ischemic) | 2.5-9.1 | 3.5-15.9 | 7.4-12.0 | 4.0-7.2 |
| | LCCA | 4.0 | 3.9 | 3.9 | 2.7 |
| | (non-ischemic) | 1.8-13.0 | 1.7-9.6 | 3.1-5.6 | 2.4-3.9 |
| Phosphoglycerides | | | | | |
| epi | LAICA | 30.5 | 28.8 | 29.7 | 31.1 |
| | (ischemic) | 29.0-35.3 | 24.2-33.0 | 29.3-31.1 | 27.5-32.3 |
| | LCCA | 32.3 | 27.4 | 28.7 | 28.4 |
| | (non-ischemic) | 29.6-39.6 | 25.4-34.2 | 27.5-30.1 | 27.1-30.1 |
| endo | LAICA | 34.6 | 28.0 | 28.7 | 28.3 |
| | (ischemic) | 33.1-38.0 | 26.2-30.3 | 25.8-30.7 | 25.6-31.1 |
| | LCCA | 35.9 | 28.2 | 29.7 | 30.4 |
| | (non-ischemic) | 33.6-38.0 | 24.7-31.5 | 27.1-31.1 | 27.3-31.8 |

Table 7.5 Contents of ATP, creatine phosphate and glycogen ($\mu$mol.g$^{-1}$ dry weight) in the LAICA and LCCA perfusion area in animals without (control, n=10) and with stenosis of the LAICA during 10 (n=10), 60 (n=10), and 120 min (n=10). Median values and 95% limits are shown.

| | | Control | Ischemia | | |
|---|---|---|---|---|---|
| Time (min) | | | 10 | 60 | 120 |
| ATP | | | | | |
| epi | LAICA | 21 | 13 | 10* | 11* |
| | (ischemic) | 13-26 | 11-16 | 6-16 | 5-15 |
| | LCCA | 17 | 16 | 18 | 20 |
| | (non-ischemic) | 10-24 | 13-22 | 11-20 | 0-23 |
| endo | LAICA | 17 | 13* | 6* | 5* |
| | (ischemic) | 12-19 | 10-15 | 4-12 | 3-13 |
| | LCCA | 18 | 18 | 16 | 17 |
| | (non-ischemic) | 13-20 | 16-20 | 15-20 | 12-23 |
| Creatine phosphate | | | | | |
| epi | LAICA | 41 | 20* | 15* | 29* |
| | (ischemic) | 26-54 | 10-30 | 10-22 | 12-36 |
| | LCCA | 48 | 32 | 45 | 41 |
| | (non-ischemic) | 26-61 | 23-67 | 27-53 | 26-53 |
| endo | LAICA | 37 | 16*o | 11*o | 18*o |
| | (ischemic) | 29-53 | 10-35 | 9-23 | 12-22 |
| | LCCA | 39 | 38 | 34o | 41 |
| | (non-ischemic | 25-51 | 20-59 | 22-43 | 31-48 |
| Glycogen | | | | | |
| epi | LAICA | 170 | 180 | 90* | 100* |
| | (ischemic) | 105-205 | 45-160 | 70-145 | 60-165 |
| | LCCA | 165 | 140 | 150 | 160 |
| | (non-ischemic | 145-225 | 110-190 | 130-220 | 125-215 |
| endo | LAICA | 150 | 100*o | 80*o | 60*o |
| | (ischemic) | 130-180 | 45-165 | 40-170 | 35-120 |
| | LCCA | 165 | 150 | 145 | 160 |
| | (non-ischemic) | 145-190 | 75-180 | 125-230 | 130-205 |

* $p < 0.05$ as compared with the content in the LCCA (non-ischemic) area.
o $p < 0.05$ as compared with the outer layers of the same area.

mic area of the same heart after 10, 60 and 120 min after onset of stenosis
were lower than the values obtained during experiments without inducement of
stenosis (table 7.4). The phosphoglyceride content in the normoxic myocardium
(both areas in the control experiments and the LCCA area in the experiments
where ischemia was induced) was higher in the inner than in the outer layers.
In the ischemic myocardium, however, phosphoglyceride content was lower in
the inner than in the outer layers (significant after 120 min of ischemia;
table 7.4).

### 7.3.4 Time course of myocardial content of ATP, creatine phosphate and glycogen during ischemia

In the ischemic area ATP content was significantly decreased in the middle
and inner layers of the ischemic area as compared to the non-ischemic area
after 10 min of ischemia, and subsequently decreased further in all layers
onto 120 min of ischemia. After 10, 60, and 120 min of ischemia, ATP content
tended to be lower in the inner than in the outer layers, but this difference
was not statistically significant (table 7.5).

After the rapid decline in creatine phosphate contents during the first 5
min of ischemia (chapter 6), these contents further decreased to approximate-
ly 30% of the values in the non-ischemic area (table 7.5). However, in the
second hour after the onset of stenosis the creatine phosphate content signi-
ficantly increased in all three layers. At all sample times, the contents in
the inner layers of the ischemic area were significantly lower than those in
the outer layers (table 7.5).

In the inner and middle layers no significant decrease in glycogen content
was found  before 10 min of ischemia and in the outer layers not before 60
min of ischemia (chapter 6, table 7.5). The values in the inner layers were
significantly lower than those in the outer layers at 10 and 120 min, but not
at 60 min. After 120 min of ischemia, glycogen content had decreased to 63%
(outer layers) and 38% (inner layers) of the values in the non-ischemic area
(table 7.5).

### 7.3.5 Time course of biochemical variables in the blood

The arterial concentrations of glucose, lactate, inorganic phosphate and
oxygen content did not significantly change during the experimental period.
The arterial NEFA concentration was temporarily increased after 10 min and
the arterial potassium concentration showed an increase of 14% after 60 and

Table 7.6 Arterial concentrations (mM), AV differences (mM) and net uptake ($\mu$mol.g$^{-1}$.min$^{-1}$) of oxygen, substrates, inorganic phosphate and potassium before and during 120 min of ischemia. Median values and 95% limits are shown.

| Time (min) | -5 | 10 | 60 | 120 |
|---|---|---|---|---|
| **Oxygen** | | | | |
| Arterial | 7.4 | 7.5 | 7.4 | 7.7 |
| | 6.8-7.9 | 7.1-7.9 | 6.4-7.9 | 5.9-8.8 |
| AV diff. | 4.7 | 5.9* | 5.5* | 5.8* |
| | 4.1-5.5 | 4.9-6.5 | 4.7-6.6 | 3.9-6.8 |
| Uptake | 3.4 | 1.3* | 1.3* | 1.1* |
| | 2.8-4.5 | 0.9-1.7 | 0.9-1.6 | 0.5-2.0 |
| **Glucose** | | | | |
| Arterial | 5.9 | 6.0 | 5.8 | 6.1 |
| | 5.5-6.1 | 5.5-6.5 | 5.3-6.5 | 4.4-8.4 |
| AV diff. | 0.1 | 0.7* | 1.1* | 1.1* |
| | (-0.2)-0.3 | 0.0-1.2 | 0.6-1.7 | 0.5-3.2 |
| Uptake | 0.1 | 0.2* | 0.3* | 0.2 |
| | (-0.1)-0.2 | 0.0-0.4 | 0.1-0.3 | 0.1-0.7 |
| **Lactate** | | | | |
| Arterial | 1.7 | 1.7 | 1.7 | 1.5 |
| | 1.3-1.8 | 1.4-2.0 | 1.3-2.0 | 0.9-2.3 |
| AV diff. | 0.49 | -1.67* | -1.07* | -0.63* |
| | 0.25-0.74 | (-2.81)-(-0.67) | (-1.74)-(-0.49) | (-0.96)-0.23 |
| Uptake | 0.35 | -0.37* | -0.25* | -0.09* |
| | 0.19-0.55 | (-0.58)-(-0.16) | (-0.39)-(-0.15) | (-0.23)-0.11 |
| **NEFA** | | | | |
| Arterial | 0.24 | 0.30* | 0.28 | 0.25 |
| | 0.16-0.30 | 0.21-0.47 | 0.21-0.40 | 0.09-0.53 |
| AV diff. | 0.11 | 0.17* | 0.16 | 0.08 |
| | 0.04-0.19 | 0.04-0.28 | 0.05-0.29 | 0.05-0.24 |
| Uptake | 0.09 | 0.04* | 0.04* | 0.03* |
| | 0.02-0.12 | 0.00-0.07 | 0.02-0.06 | 0.01-0.06 |
| **Inorganic phosphate** | | | | |
| Arterial | 1.73 | 1.79 | 1.85 | 1.91 |
| | 1.62-2.01 | 1.75-2.10 | 1.67-1.98 | 1.74-2.22 |
| AV diff. | -0.03 | -0.69* | -0.13* | -0.10 |
| | (-0.09)-0.00 | (-0.47)-(-1.08) | (-0.24)-(-0.04) | (-0.24)-0.00 |
| Uptake | -0.03 | -0.16* | -0.03 | -0.03 |
| | (-0.05)-0.00 | (-0.23)-(-0.08) | (-0.04)-(-0.01) | (-0.06)-0.00 |
| **Potassium** | | | | |
| Arterial | 3.70 | 3.95* | 4.25* | 4.20* |
| | 3.55-3.90 | 3.70-4.15 | 3.90-4.50 | 3.75-5.00 |
| AV diff. | -0.01 | -1.06* | -0.14 | -0.15 |
| | (-0.18)-0.17 | (-1.65)-(-0.45) | (-0.40)-0.15 | (-0.45)-0.55 |
| Uptake | -0.01 | -0.22* | -0.03 | -0.03 |
| | (-0.16)-0.10 | (-0.31)-(-0.13) | (-0.04)-(-0.02) | (-0.07)-0.06 |

* $p < 0.05$ as compared with t= -5 min

120 min as compared to time -15 min (table 7.6).

Inducement of ischemia reduced oxygen uptake (calculated by multiplying the arterio-local venous (AV) differences and mean blood flow in the ischemic area; section 3.7) by approximately 65% as compared to the pre-ischemic situation (table 7.6). The AV differences of glucose increased 15-fold, causing a 4.5-5 fold increase in net glucose uptake after 10 to 120 min of ischemia. Lactate was released during ischemia in a biphasic pattern (table 7.6). Net release of lactate was maximal at 10 min after onset of ischemia. Thereafter, this release gradually diminished but remained present in the majority of the experiments after 120 min of stenosis (table 7.6). Although the AV differences of NEFA increased, net uptake of these substances decreased by 45% (after 10 and 60 min) and 65% (after 120 min) as compared with the pre-ischemic values (table 7.6). During ischemia, the AV differences for triacylglycerol were not significantly different from those during normoxia (data not shown). In the first 10 min of the ischemic period, the release of inorganic phosphate and potassium paralleled that of lactate (chapter 6) but at 60 and 120 min the release of the former two substances was not significantly different from the pre-ischemic values (table 7.6).

### 7.3.6 Time course of regional mechanical variables

.As shown in chapter 6, regional shortening in the ischemic area ceased within the first minute after coronary artery stenosis. Before time 10 min, circumferential ($e_c$) and base-to-apex ($e_z$) were already close to zero and the values of these variables did not change significantly during the remaining experimental period (table 7.7). Ischemic values of $\gamma$ were about half the normoxic values and were approximately constant between 10 and 120 min after the onset of stenosis.

In chapter 5, it has been shown that information about differences in shortening between the inner and outer layers of the myocardial wall can be obtained from epicardial minimal ($e_{min}$) and maximal shortening ($e_{max}$) as calculated from $e_c$, $e_z$, and $\gamma$ (section 3.3.2), because the orientation of $e_{min}$ and $e_{max}$ approximates the fiber directions in the inner and outer layers, respectively. Both calculated variables decreased substantially within 1 min of ischemia (chapter 6) but only slightly thereafter. The negative values of $e_{min}$ indicate lengthening during the ejection phase in the direction of the fibers in the inner layers. This lengthening tended to increase during the experimental period but this increase was not significant. The small

Table 7.7 Regional shortening, shear and segment length before and
during 120 min of stenosis. Median values and 95% limits are
shown.

| Time (min) | -5 | 10 | 60 | 120 |
|---|---|---|---|---|
| Circumferential shortening (%) | 5.2<br>3.9-7.3 | 0.2*<br>(-0.3)-0.6 | 0.2*<br>(-0.6)-0.3 | 0.0*<br>(-1.1)-0.0 |
| Base-to-apex shortening (%) | 4.8<br>2.4-6.0 | 0.4*<br>(-0.6)-1.0 | -0.4*<br>(-0.5)-0.0 | -0.8<br>(-1.7)-0.0 |
| Shear angle (rad) | 0.06<br>0.04-0.08 | 0.03*<br>0.01-0.05 | 0.02* **<br>0.01-0.02 | 0.03*<br>(-0.01)-0.07 |
| Minimal shortening (%) | 3.7<br>1.7-4.9 | -0.6*<br>(-1.1)-0 | -0.6*<br>(-0.9)-(-0.3) | -1.2*<br>(-0.2)-(-0.7) |
| Maximal shortening (%) | 6.5<br>5.3-8.0 | 0.9*<br>0-2.5 | 0.1* **<br>(-0.2)-0.3 | 0.0* **<br>(-1.1)-1.5 |
| Begin ejection base-to-apex segment length (%) | 100.0<br>- | 108.3*<br>105.0-112.0 | 110.1*<br>106.1-111.9 | 110.6*<br>101.0-115.2 |
| Begin ejection circumferential segment length (%) | 100.0<br>- | 109.5*<br>106.0-114.8 | 109.8*<br>106.0-113.8 | 111.8<br>85.5-115.1 |

* p<0.05 as compared with t= -5 min
** p<0.05 as compared with t=10 min

positive median value of $e_{max}$, indicating the presence of residual shortening approximately along the epicardial fiber direction, after 10 min of ischemia disappeared in the course of the experimental period ($p<0.05$) (table 7.7).

At the onset of the ejection phase the distance between the coils had increased by about 10% between 10 and 120 min of ischemia as compared with the control situation, in both circumferential and base-to-apex direction.

## 7.3.7 The relation between NEFA content and the blood flow and ATP content in myocardial tissue

In fig 7.4 NEFA content and MBF after 120 min of ischemia are plotted against each other. In the ischemic area no increase in NEFA content was observed in samples, which blood flow in their vicinity was more than 0.3 $ml.min^{-1}.g^{-1}$ (which are mainly samples from the outer layers). Samples with a blood flow below this value almost invariably show an increase in NEFA content. Extreme increases in NEFA content (up to 20 times the normoxic values) are predominantly observed in samples where blood flow is below 0.1 $ml.min^{-1}.g^{-1}$.

After 60 min of ischemia, high NEFA contents did not coincide with low ATP contents in the outer and middle layers, but high NEFA contents were associated with low ATP contents in the inner layers (data not shown). The relation between ATP and NEFA content after 120 min of ischemia in all three myocardial layers is shown in fig 7.5. Increased NEFA contents were present at ATP contents below 10 $\mu mol.g^{-1}$ dry weight.

## 7.4 Discussion

### 7.4.1 Effect of ischemia on global and regional myocardial function

Stenosis of the LAICA caused ischemia in its perfusion area. This is indicated by the decrease in myocardial blood flow (MBF) and oxygen consumption in this area as well as by the release of lactate, inorganic phosphate and potassium from the affected myocardium. Release of lactate indicates anaerobic glycolysis (Williamson, 1966). Inorganic phosphate release is associated with breakdown of energy-rich phosphates (Opie et al, 1972). Potassium release is ascribed to disturbances of ion exchange across the cell membrane due to energy shortage (Owen et al, 1970).

The early loss of mechanical function of the ischemic area (chapter 6) leads to significant changes in general hemodynamic variables such as heart

Fig 7.4
Relation beween the content of NEFA and myocardial blood flow (MBF) in samples from ischemic and non-ischemic myocardium after 120 min of stenosis of the LAICA. MBF was determined in tissue from the direct vicinity of the site where samples for the determination of NEFA content were taken. Median values and 95% limits of all three myocardial layers are shown, while the figures depict the number of observations. The shaded area shows the 95% limits of the data from all non-ischemic samples.



Fig 7.5
Relation between the contents of NEFA and ATP in separate biopsies taken from ischemic and non-ischemic myocardium after 120 min of stenosis of the LAICA. For explanation of the symbols, see fig. 7.4.

rate, mean aortic pressure, $dp_{lv}/dt$ max, $p_{lved}$, and cardiac output. A more pronounced deterioration of global hemodynamics may have been prevented by the compensatory increase in heart rate and thus in mechanical function of the non-ischemic myocardium. This is indicated by a transient increase in MBF found between 1 and 10 min after onset of ischemia (see also section 6). The increased non-ischemic MBF is in agreement with findings of Driscoll and Eckstein (1967) and Redding and Rees (1968), but not with the data presented by Marcus et al (1976). Also, the decreased triacylglycerol content in the non-ischemic area points towards an increased need for more combustible substrates for energy production (Crass et al, 1971; Van der Vusse et al, 1982).

During the last hour of the experimental period, mean aortic pressure decreased. It is unlikely that these hemodynamic changes are caused by ischemia since the same phenomenon is observed in the experiments with two hours of normal perfusion. This decrease in aortic pressure is ascribed to the anesthesia used in the present study.

This decrease in aortic pressure is associated with a decrease in $dp_{lv}/dtmax$ and stroke volume indicating decreases in preload, afterload and stroke work. A lower energy demand of the left ventricle due to these changes is also suggested by the tendency of myocardial blood flow to decrease in the non-ischemic area at 120 min as compared with the previous sample times (table 7.2), which was also observed in the control experiments (chapter 4). Since blood flow in the ischemic myocardium hardly changed between 10 and 120 min this could imply that oxygen shortage in the ischemic area after 120 min is less than after 60 min of stenosis, possibly exerting effects on metabolism in the ischemic myocardium (section 7.4.3).

The relatively small changes in regional shortening between 10 and 120 min of ischemia is in agreement with data obtained in the baboon (Crozatier et al, 1978). Assessment of shortening in directions, close to the fibers in the inner and outer layers, shows that between 10 and 120 min shortening decreases slightly more pronounced in the outer than in the inner layers. This suggests that during this phase of ischemia, transmural differences in shortening gradually decrease while the transmural gradients in energy rich phosphate and glycogen contents remain unchanged (section 7.4.3) and the transmural blood flow gradient becomes even steeper (section 7.4.2).

### 7.4.2 Effect of ischemia on myocardial blood flow

Stenosis of the LAICA induced a fall in perfusion pressure and blood flow

in its perfusion area. The virtually unchanged mean transmural blood flow in
the ischemic area during the first 2 hours is in contrast to the findings of
other investigators studying the effect of coronary artery occlusion in the
dog (Redding and Rees, 1968; Marcus et al, 1976). These investigators found a
significant increase in MBF in the ischemic area, caused by early collateral
development. These different observations might be caused by differences in
collateral development after occlusion or stenosis. An alternative possibili-
ty is that the developed collateral flow is not noticed in our set-up since
we do keep the perfusion pressure constant.

The relative underperfusion of the inner layers of the ischemic myocardium
is caused by the higher intramyocardial pressure in these layers as compared
to the outer layers (Arts et al, 1979; Hoffman, 1979). Despite the unchanged
mean transmural blood flow, a steady decrease in endo/epicardial flow ratio
during the experimental period was found. This is consonant with findings of
other investigators that the early increase in ischemic blood flow (see
above) is mainly in the outer layers (Hirzel et al, 1976; Marcus et al, 1976;
Rivas et al, 1976; Capurro et al, 1979). The distribution of myocardial blood
flow away from the inner layers is in agreement with the theory that during
ischemia blood flow is redistributed from the necrotic inner to the surviving
outer layers (Hirzel et al, 1976).

## 7.4.3 Effect of ischemia on myocardial metabolism

The large AV differences of glucose and the hardly affected AV differences
of NEFA during ischemia are in agreement with findings of other investigators
(Opie et al, 1973). The greater role of glucose catabolism during ischemia as
compared to normoxia is shown by the increase in net uptake of glucose (pre-
sent study) and by the increase in glucose oxidation relative to that of NEFA
(Opie et al, 1973).

From the present results it is not clear, whether transmural differences
in glucose uptake exist, since the contribution of each myocardial layer to
the measured AV differences is not known. Glycogenolysis is mostly enhanced
in the inner layers. This may be explained by a higher phosphorylase activity
in the inner than in the outer layers because concentrations of AMP, inorga-
nic phosphate and glucose-6-phosphate are higher in the former layers (Opie
et al, 1976; Higuchi et al, 1981). A higher phosphorylase a and b activity in
the inner than in the outer layers has been measured by Ichihara and Abiko
(1975).

Metabolic improvements occurring between 10 and 120 min of ischemia are suggested by the increased creatine phosphate contents and the decreased net release of lactate, potassium and inorganic phosphate. This might be caused by the anesthesia-induced decrease in left ventricular inotropy (section 7.4.1). However, 30 min after coronary artery occlusion increasing creatine phosphate contents have been reported by Ichihara and Abiko (1975), and Opie et al (1973) showed that the release of the three above-mentioned substances decreased between 10 and 120 min after occlusion. A decreased energy demand due to diminisched mechanical function is not likely since shortening in the ischemic area hardly changed between 10 and 120 min of ischemia. A cause of this apparent metabolic improvement might be redistribution of blood flow within the ischemic area. Tissue damage has been shown to be widely hetero-geneous within the ischemic tissue: damaged and reasonably preserved cells are present in close proximity of one another (Schaper et al, 1979; Vasdev et al, 1980; Van der Vusse and Frederik, unpublished results). Redistribution of blood flow to the surviving cells might improve the metabolic situation of these cells, because initially all cells competed for the same reduced oxygen supply.

Between 10 and 120 min of ischemia, metabolism in the inner layers is more affected than in the outer layers, as shown by the lower contents of creatine phosphate and glycogen in the inner layers. This is in agreement with the findings of Griggs et al (1972), Opie et al (1975), Opie and Owen (1976), and Higuchi et al (1981). The absence of a significant transmural gradient in ATP content is in agreement with the findings of Griggs et al (1972) after a relatively short period of ischemia and with those of Higuchi et al (1981) who observed relatively moderate transmural differences in ATP content only after 15 min of severe, but not after 15 min of mild ischemia.

### 7.4.4 Effect of ischemia on myocardial content of fatty acids

As previously mentioned (Van der Vusse et al, 1981b, 1982) the myocardial NEFA content during normoxia and ischemia as found in our laboratories are much lower than those reported by others (Weglicki et al, 1973; Haider et al, 1977; Weishaar et al, 1977 and 1979; Andrieu et al, 1979, 1980). These diffe-rences are likely due to differences in the assay method of NEFA (Christie, 1973; Kramer and Hulan, 1978; Van der Vusse et al, 1980). Evidence is present that values of tissue NEFA concentrations as found in the present study are the closest approximation to the actual myocardial content (Van der Vusse et

al, 1981b, 1982) which is supported by data of Hunneman et al (1981), who recently reported similar low values of NEFA in the normoxic dog heart.

In the present study it is shown that tissue contents of NEFA are not increased after 10 min of ischemia. However, significant accumulation of NEFA is observed after 60 and 120 min of ischemia. NEFA accumulation shows remarkable differences between the various layers with respect to time course, composition and amount of accumulated NEFA, and the relation with ATP content. This suggests that the accumulated NEFA in the various layers are derived from different sources.

While NEFA content in the inner layers increase with prolongation of ischemia, NEFA accumulation in the outer layers exhibit a maximum increase after 60 min of ischemia (fig 7.2). The accumulation of the various individual fatty acids are different in these two layers (fig 7.3).

In all layers initially no evident relation existed between ATP and NEFA contents, whereas this was clear in the inner layers after 60 min and in all layers after 120 min of ischemia. At the latter time interval, NEFA contents proved to be increased at ATP contents below 10 $\mu$mol.g$^{-1}$ dry weight. These findings are interesting, since Hearse and Stewart (1974) showed that mechanical recovery after global ischemia was impaired at similar ATP contents. ATP contents below 5 $\mu$mol.g$^{-1}$ dry weight appeared to be related to impaired volume regulation and resynthesis of ATP after reoxygenation (Reimer et al, 1981).

In the present study, the median values of triacylglycerol content in the outer ischemic layers were lower as compared with the content in the normoxic LAICA perfusion area but this difference did not reach the level of significance (table 7.4). Possible changes may be masked by the large variation in triacylglycerol contents in the outer layers (even within one animal).

### 7.4.5 Possible sources of NEFA accumulation in the outer layers after 60 min of ischemia

In the outer layers, individual fatty acids with the highest relative increase after 60 min of ischemia are oleic and linoleic acid. Potential sources of these fatty acids are NEFA and triacylglycerol in the blood and triacylglycerol and phosphoglycerides in tissue, which are rich in these substances. The following mechanisms may be involved in the accumulation of NEFA in the outer layers.

- During ischemia, the arterio-local venous differences of NEFA across the affected area remain positive, indicating that uptake of NEFA continues to occur. Since fatty acid oxidation is impaired under ischemic conditions (Opie et al, 1973; Liedtke et al, 1978; Shug et al, 1978), part of the NEFA extracted from the blood, with oleic acid as predominant fatty acid (Rothlin and Bing, 1961; Van der Vusse et al, 1982), might be responsible for the accumulated NEFA. Because of the measured inhomogeneities in ischemic myocardial blood flow, the uptake of substrates across the ischemic tissue might vary from layer to layer. Although the higher blood flow in the outer than in the inner layers might indicate that the AV differences predominantly reflect metabolism in the outer layers, we cannot definitely conclude whether the observed extraction and tissue accumulation of NEFA occurs in the same layer or cells of the tissue where NEFA content is increased.
- Uptake of triacylglycerol from the blood has been found to be not statistically significant and thus would not contribute to the accumulation of NEFA. However, even an uptake of this substance within the measuring error (2%) of the arterio-local venous differences throughout the ischemic period could be responsible for the measured increased NEFA contents in the subepicardium.
- Lipolyis of phosphoglycerides may not be excluded, since significant accumulation of arachidonic acid is observed and phosphoglycerides are rich in this substance (Van der Vusse et al, 1982).
- The accumulation might also be due to lipolysis of myocardial triacylglycerol. The effect of triacylglycerol lipolysis should be reflected in decreased contents of triacylglycerol. The measured NEFA accumulation, however, results from less than 1% lipolysis of the total amount of triacylglycerol so that this mechanism can be easily missed. Under ischemic conditions, lipolysis is indicated by the dilution of $^{14}$C-labeled palmitate in local venous blood with cold NEFA (Opie et al, 1973) and the glycerol release from the ischemic myocardium (Vik-Mo et al, 1979). Moreover, Jesmok et al (1978) showed that 30 min after coronary artery occlusion triacylglycerol content had decreased in the outer layers. It should be mentioned that it is not known whether this lipolysis occurs in myocytes or in the epicardial fat.

One should realize that the relative increase of the individual fatty acids does not correspond completely with one of the above-mentioned sources.

Therefore, several processes may be involved contemporarily.

Lipolysis during ischemia is thought to be caused by release of endogenous catecholamines under ischemic conditions (Wollenberger and Shahab, 1967; Hough and Gevers, 1975). Indeed, Jesmok and coworkers (1978) showed that the early lipolysis could be blocked with a heart-specific beta-receptor blocking agent.

After 60 min of ischemia, lipolysis of triaglycerol dominates synthesis of this substance to a larger extent in the outer layers, than in the inner ones, as suggested by the lower NEFA contents in the latter layers. This is in agreement with the rather increased than decreased triacylglycerol content in the inner layers in the study of Jesmok et al (1978). The more severe degree of ischemia in the inner layers causes, among others, high glycerol-3-phosphate concentrations. High concentrations of this substance may promote incorporation of NEFA into triacylglycerol (Neely and Morgan, 1974; Opie, 1976).

## 7.4.6 Possible sources of NEFA accumulation in the inner layers

In the inner layers, the individual fatty acids with the highest relative increase are arachidonic and linoleic acid. The source of linoleic acid may be exogenous or endogenous (see above). Since arachidonic acid is not taken up from the blood in measurable amounts (Van der Vusse et al, 1982) during ischemia, an endogenous source for at least a part of the accumulated NEFA seems feasible. The most likely candidates are myocardial phosphoglycerides, rich in arachidonic and linoleic acid (Van der Vusse et al, 1982). This is supported by the observation of Vasdev and coworkers (1980) that the individual phosphoglyceride classes in sarcolemmal and mitochondrial fractions isolated from ischemic myocardium are significantly decreased. This phosphoglyceride lipolysis may be induced by phospholipase $A_2$ which is stimulated by high $Ca^{2+}$ concentrations (Franson et al, 1978). During ischemia high $Ca2^+$ concentrations are suggested to be present within the cells because the $Ca^{2+}$ homeostasis is impaired by ATP depletion (Nayler, 1981).

Weglicki et al (1973) indeed suggested phospholipase $A_2$ to be active during ischemia. Increased levels of the products of the phospholipase $A_2$-phosphoglyceride interaction (i.e. lysophosphoglycerides) have been shown to be present by Sobel et al (1978). These findings support the hypothesis that at least a part of the NEFA, accumulated in the inner layers, is the result of phosphoglyceride lipolysis. The not significant difference in phosphogly-

ceride content between the ischemic and non-ischemic tissue in the same
animal is not contradictory to this hypothesis, since lipolysis of only 1% of
the phosphoglycerides may result in a 30-fold increase in NEFA content.

It should be noticed that on the base of the present data it cannot be
concluded whether the above-mentioned NEFA accumulation occurs in the myo-
cytes or in other cells of the myocardium. Using an isolated heart prepara-
tion, which allowed the separate collection of coronary effluent and inter-
stitial effluent, Stam and Huelsmann (1981) showed that hormone sensitive
release of lysophosphatidylcholine was mainly recovered in the coronary
effluent whereas products from triacyl-glycerol lipolysis were present in the
interstitial effluent. These data suggest that myocardial lysophosphatidyl-
choline formation under these (non-ischemic) conditions took place at the
level of the coronary vasculature rather than in the myocytes.

### 7.4.7 Discussion on the harmful effects of NEFA accumulation

Several harmful effects during ischemia have been ascribed to high intra-
cellular NEFA concentration as well as high acylCoA and acylcarnitine concen-
trations (section 2.3.3).

During ischemia in vivo glycolytic inhibition by high NEFA concentrations
as reported to occur in vitro (Lea and Weber, 1968; Ramadoss et al, 1976),
does not seem to be influenced by extra or intracellular NEFA: net uptake of
glucose remains constant from 10 to 120 min at a level 5 times the pre-ische-
mic value. No convincing evidence is present, that the decreased lactate
release after 10 min of ischemia might be caused by glycolytic inhibition,
since redistribution of blood flow to surviving cells and a decreased oxygen
shortage in the ischemic area might cause this phenomenon (see above). Yet no
support has been found for the theory that high cellular NEFA concentrations
cause premature glycogen depletion (Cowan and Vaughan Williams, 1980). Glyco-
gen breakdown started before the onset of NEFA accumulation and the majority
of the glycogen was metabolized during the first hour of ischemia.

A proposed ATPase stimulation by increased NEFA concentrations (Pressman
and Lardy, 1956; Borst et al, 1962), is in contradiction with the findings in
the present study, after 60 min, ATP concentrations did not decrease after
the first hour of ischemia, even not in the outer layers where the highest
NEFA concentrations were present after 60 min. A restriction should be made
in the case that low ATP and high NEFA contents do not occur in the same
cell.

The harmful effect of high NEFA concentrations on mechanical function of the ischemic heart (Katz and Messineo, 1981; Feuvray and Plouet, 1981) is not probable since early mechanical failure occurs within one min after onset of ischemia (chapter 6), whereas after 10 min of ischemia no change in NEFA contents could be observed. Moreover, myocardial wall shortening hardly diminishes when NEFA contents increases.

## 7.5 Conclusions

- Stenosis of the LAICA resulted in significantly impaired global hemodynamics after 10 min of ischemia. Only slight changes in the hemodynamic variables occurred in the following 110 min of ischemia except for a decrease in aortic pressure and $dp_{lv}$/dtmax which was probably caused by the anesthesia used.
- Increased MBF (after 10 min of ischemia) and decreased triacylglycerol content of the non-ischemic area suggest increased workload in this area to compensate for function loss of the jeopardized myocardium.
- Maintenance of a constant low perfusion pressure to the ischemic area resulted in a virtually unchanged mean transmural flow to the ischemic area between 10 and 120 min.
- At a longer duration of ischemia, the relative underperfusion of the inner layers became more severe.
- Contents of NEFA in ischemic myocardium do not increase before 10 min of ischemia.
- In the outer layers, NEFA content transiently increased after 60 min of ischemia, whereas in the inner and middle layers NEFA content gradually increased from 60 to 120 min.
- The highest relative increase of arachidonic and linoleic acid in the inner and middle layers suggest (substantial) lipolysis of phosphoglycerides.
- The accumulation of NEFA occurred when myocardial blood flow was below 0.3 $ml.min^{-1}.g^{-1}$, and ATP contents were below $10 \mu mol.g^{-1}$ dry weight.
- The changes in mechanical function and metabolism in the ischemic myocardium are not necessarily due to NEFA accumulation.

8. THE EFFECT OF ELEVATED ARTERIAL NON-ESTERIFIED FATTY ACID CONCENTRATIONS ON HEMODYNAMICS AND MYOCARDIAL METABOLISM AND BLOOD FLOW DURING ISCHEMIA[*]

## 8.1 Introduction

High arterial non-esterified fatty acid (NEFA) concentrations are consi-
dered to be harmful for the ischemic myocardium (Kurien and Oliver, 1966;
Oliver et al, 1968; Takano, 1976). In these investigations positive relations
were found between arterial NEFA levels on the one hand and infarct size,
ventricular arrhythmias and ventricular fibrillation on the other. In other
studies, however, these relations could not be confirmed (Rutenberg et al,
1969; Most et al, 1974; Dagenais and Jalbert, 1977).

Although the occurrence of arrhythmias and of left ventricular failure in
relation to elevated plasma NEFA levels has been studied extensively, only
limited information is available about myocardial metabolism and regional
myocardial blood flow under these circumstances, especially during ischemia.

In this chapter, carbohydrate, NEFA and oxygen utilization of the ischemic
myocardium as well as the release of potassium and inorganic phosphate from
this tissue at normal and elevated arterial NEFA levels were studied in
anesthetized dogs. Left ventricular hemodynamics and regional myocardial
blood flow were measured as well. Myocardial ischemia was induced by partial
occlusion (stenosis) of the LAICA as described in section 3.1.4. NEFA concen-
trations were elevated by intravenous injection of heparin, intralipid or a
combination of these substances (intralipid-heparin).

## 8.2 Experimental design

### 8.2.1 Protocol

The dogs were allotted to 3 groups of 8 animals each. In each group
the same protocol was followed during the control period and the first
60 min of stenosis. Thereafter, heparin (5000 I.U.) was injected intrave-

[*]Has been publisched in Basic Res. Cardiol. 76, 197-210 (1981)

Fig 8.1 Protocol of the experiments. H=heparin 5000 I.U. i.v., I=in-
tralipid 50 ml 20% i.v., TM= injection of radioactive micro-
spheres. The dashes on the time axis represent the sample time
for biochemical and hemodynamical variables.

Table 8.1 Relative changes in total myocardial blood flow (MBF) and
endo/epicardial blood flow ratio in ischemic and non-ische-
mic tissue after elevation of arterial FFA concentrations
expressed as percentage of the value before injection of the
substances.

| Variable | Non-ischemic | | Ischemic | |
|---|---|---|---|---|
| | Intralipid | Intralipid + Heparin | Intralipid | Intralipid + Heparin |
| Total MBF | 13* 5-22 | 11 (-9)-27 | -10 (-8)-33 | -16* (-45)-0 |
| Endo/epi-cardial blood flow ratio | -1 (-1)-2.3 | -3 (-7)-6 | -3 (-11)-13 | -13* (-52)-0 |

* $p < 0.05$

nously in group I, whereas in group II and III respectively intralipid (50 ml 20% I.V., Vitrum, Stockholm) and intralipid-heparin were administered. The last procedure is comparable with the method of elevating arterial NEFA concentrations used by Opie and co-investigators (1973). Both in group II and III, the compounds were injected slowly (within 1-2 min). Arterial and local venous blood samples were taken 15 min, just before, and 10, 20, 30, 45, 60, 70, 80, 90, 105, and 120 min after inducement of the stenosis. The continuously recorded hemodynamic variables and cardiac output were also determined at these moments (fig 8.1). In group II and III, regional myocardial blood flow (MBF) was determined just before and 20 min after administration of intralipid and intralipid-heparin, respectively, using tracer microspheres as described in section 3.4.

## 8.2.2 Data analysis

To compare the hemodynamics of the three groups just before onset of ischemia, differences between the values of heart rate and mean aortic pressure at time 0 min were evaluated for statistical significance by applying Wilcoxon's two-sample test.

Information about the effect of heparin, intralipid or the intralipid-heparin during myocardial ischemia was obtained by comparison of the data within each animal. Influences on the hemodynamic and biochemical variables were evaluated by comparing the values at time 70, 80, 90, 105 and 120 min with those just before injection of the substances at time 60 min. Information about the effect on MBF was obtained by comparing the values just before (time 60 min) and 20 min after injection of the substances. Differences between the values of the various variables were evaluated for statistical significance by applying Wilcoxon's matched-pairs signed-ranks test (two-tailed probability). The data are presented as median values and 95% confidence limits. A value of $p < 0.05$ was considered to be a significant difference.

## 8.3 Results

### 8.3.1 Hemodynamic variables

At time 0 min (before onset of stenosis) the heart rate and mean aortic pressure were for group I, II and III: 110 (60-160), 135 (79177), and 85

Table 8.2 Arterial values of various biochemical variables before and
20 min after administration of heparin, intralipid or both
substances.

| Variable | HEPARIN Before | HEPARIN After | INTRALIPID Before | INTRALIPID After | HEPARIN + INTRALIPID Before | HEPARIN + INTRALIPID After |
|---|---|---|---|---|---|---|
| NEFA (mmol/l) | 0.20 0.16-0.32 | 0.30* 0.24-0.42 | 0.33 0.27-0.37 | 0.53* 0.38-0.85 | 0.17 0.14-0.20 | 0.81* 0.66-1.23 |
| Lactate (mmol/l) | 1.09 0.72-1.84 | 0.92 0.72-1.75 | 1.35 0.90-1.69 | 2.31* 1.77-3.30 | 1.70 1.40-3.18 | 3.43* 2.33-4.72 |
| pH | 7.43 7.41-7.45 | 7.43 7.41-7.45 | 78.39 7.36-7.41 | 7.37* 7.34-7.40 | 7.44 7.42-7.48 | 7.41* 7.39-7.46 |
| $pCO_2$ | 3.9 3.3-3.4 | 3.9 3.5-4.0 | 4.4 4.0-4.5 | 4.3 4.1-4.4 | 3.7 3.2-3.9 | 3.6 3.3-5.7 |

* $p < 0.05$



Fig 8.2 Time course of arterial NEFA concentrations in the three
experimental groups. The median values and 95% limits are
presented. The arrow indicates the injection of the substan-
ces. ●=heparin (group I). ■=intralipid (groupII). ▲=intra-
lipid-heparin (groupIII).
* $p < 0.05$ as compared with time 60 min.

Table 8.3 Myocardial blood flow ($ml.min^{-1}.g^{-1}$), endo/ epicardial blood flow ratio and the AV differences (mM) and net uptake (umol- $.min^{-1}.g^{-1}$) of various biochemical variables in the ischemic

|  | HEPARIN | |
|---|---|---|
|  | Before | After |
| MBF | - | - |
| Endo/epicardial ratio | - | - |
| NEFA | | |
| AV difference | 0.08<br>0.04-0.19 | 0.11<br>0.07-0.15 |
| net uptake | - | - |
| Glucose | | |
| AV difference | 1.5<br>1.3-2.5 | 1.5<br>0.7-1.7 |
| net uptake | - | - |
| Lactate | | |
| AV difference | -1.85<br>(-3.2)-(-0.37) | -1.11<br>(-1.39)-(-0.17) |
| net uptake | - | - |
| Oxygen | | |
| AV difference | 5.6<br>2.3-6.6 | 5.6<br>4.2-5.9 |
| net uptake | - | - |

Table 8.4 AV differences of potassium, inorganic phosphate, pH, and $pCO_2$ across the ischemic myocardium before and 20 min after

|  | HEPARIN | |
|---|---|---|
|  | Before | After |
| $K^+$ | -0.10<br>(-0.60)-0.10 | -0.37<br>(-1.25)-0.05 |
| Pi | -0.13<br>(-0.33)-(-0.03) | -0.02<br>(-0.22)-0.03 |
| pH | 0.10<br>0.07-0.15 | 0.09<br>0.05-0.13 |
| $pCO_2$ | -2.3<br>(-3.9)-(-1.7) | -3.3<br>(-3.9)-(-1.6) |

myocardium just before and 20 min after elevating NEFA concentrations
by injecting heparin, intralipid or both substances. For explanation
of the signs, see table 8.1.

| INTRALIPID | | INTRALIPID + HEPARIN | |
|---|---|---|---|
| Before | After | Before | After |
| 0.21 | 0.22 | 0.48 | 0.29* |
| 0.11-0.36 | 0.12-0.42 | 0.25-0.56 | 0.21-0.45 |
| 0.40 | 0.35 | 0.52 | 0.35* |
| 0.16-0.56 | 0.18-0.57 | 0.29-0.59 | 0.22-0.58 |
| 0.05 | -0.01 | 0.02 | 0.10 |
| (-0.14)-(-0.02) | (-0.11)-0.14 | (-0.02)-0.06 | 0.04-0.20 |
| 0.01 | 0.00 | 0.01 | 0.02 |
| 0.00-0.01 | (-0.03)-0.03 | (-0.01)-0.02 | 0.01-0.05 |
| 1.0 | 0.7* | 0.4 | 0.7 |
| 0.8-1.4 | 0.5-1.1 | 0.0-0.8 | 0.1-1.2 |
| 0.2 | 0.2 | 0.1 | 0.2 |
| 0.1-0.3 | 0.1-0.3 | 0.0-0.2 | 0.0-0.3 |
| -1.03 | -1.29 | -0.03 | -0.50* |
| (-1.73)-(-0.48) | (-3.75)-(-0.07) | (-0.23)-0.34 | (-0.95)-(-0.25) |
| -0.18 | -0.19 | 0.02 | -0.13* |
| (-0.36)-(-0.12) | (-1.56)-0.02 | (-0.06)-0.18 | (-0.31)-0.06 |
| 6.1 | 6.0 | 5.8 | 5.8 |
| 5.7-7.0 | 5.8-7.3 | 3.7-6.7 | 4.8-6.4 |
| 1.4 | 1.4* | 2.6 | 1.7* |
| 0.7-2.3 | 0.8-3.0 | 1.3-3.3 | 1.1-2.4 |

elevating arterial NEFA concentrations by injection of heparin, intralipid
or both substances. For explanation of the signs, see table 8.1

| INTRALIPID | | INTRALIPID + HEPARIN | |
|---|---|---|---|
| Before | After | Before | After |
| -0.53 | -0.50 | -0.33 | -0.35 |
| (-0.65)-(-0.10) | (-0.90)-(-0.15) | (-0.50)-(-0.15) | (-0.60)-0.00 |
| -0.34 | -0.24 | -0.11 | -0.15 |
| (-0.40)-(-0.09) | (-0.41)-(-0.13) | (-0.21)-(-0.03) | (-0.33)-(-0.05) |
| 0.09 | 0.07 | 0.07 | 0.07 |
| 0.05-0.18 | 0.06-0.11 | 0.05-0.08 | 0.06-0.09 |
| -2.1 | -2.5 | -2.1 | -1.9 |
| (-3.1)-(-1.7) | (-3.2)-(-1.6) | (-2.4)-(-1.3) | (-2.9)-(-0.09) |

(75-115) beats.min$^{-1}$, and 10.8 (8.8-12.4), 10.3 (8.5-13.2) and 10.0 (8.0-10.3) kPa, respectively. These values were not significantly different. Intravenous injection of heparin resulted in a slight and gradual decrease in systolic (median fall maximally: 1.4 kPa (=8%)) and diastolic aortic pressure (median fall maximally: 0.7 kPa (=10%)). This decrease was significant 45 and 60 min after administration of heparin. Injection of intralipid (group II) or intralipidheparin (group III) induced a transient increase in blood pressure (of 0.5-2.0 kPa) within the first min after injection. Ten min after injection these changes were not significant anymore. Heart rate gradually increased significantly after administration of heparin as well as intralipid-heparin (maximum rise: 45; 39% and 23 beats.min$^{-1}$; 23%). After administration of intralipid, the maximal rise in heart rate was only 4 beats.min$^{-1}$ (2.5%). In all three groups, this increase became significant 45 min after injection.

Heparin, intralipid or the intralipid-heparin neither induced cardiac arrhythmias nor influenced occasionally existing arrhythmias such as nodal rhythms or atrial fibrillation.

Administration of the substances had no significant effect on $dp_{lv}/dtmax$, and cardiac output.

## 8.3.2 Regional myocardial blood flow

After administration of intralipid (group II), total MBF significantly increased in the non-ischemic myocardium (median increase: 13%). In the ischemic tissue, MBF did not change significantly (table 8.1). After simultaneous injection of heparin and intralipid, total MBF did not change significantly in the non-ischemic myocardium but decreased significantly in the ischemic part (table 8.1).

In the non-ischemic myocardium, the transmural distribution of MBF was not significantly affected by the administration of intralipid or heparin and intralipid (table 8.1). In the ischemic myocardium, the transmural distribution (endo/epicardial ratio) of MBF was not significantly changed after the injection of intralipid. After the administration of heparin together with intralipid, however, the subendocardial layers in the ischemic myocardium became more underperfused as compared with the subepicardial layers in this area (table 8.1).

### 8.3.3 Biochemical variables

Intravenous injection of heparin (group I), intralipid (group II), or intralipid-heparin (group III) elevated the arterial NEFA concentrations from 0.20 to 0.30 mM, from 0.33 to 0.53 mM, and from 0.17 to 0.87 mM, respectively (table 8.2). The arterial NEFA concentrations remained constantly elevated in group I and II, but gradually decreased in group III at the end of the experimental period (fig 8.2). In each group, the concentrations 60 min after injection of the substances were still increased as compared to those before injection of the substances.

Immediately after injection of intralipid as well as intralipidheparin, the arterial lactate concentrations increased to values 2-3 times higher than the control values. Arterial $p_{CO2}$ was not affected by the elevated arterial NEFA concentrations (table 8.2).

The elevated arterial NEFA concentrations did not result in a significant change in AV differences of NEFA and glucose across the ischemic area (table 8.3). Yet the AV differences of lactate did not change in group I and II. In group III, however, the AV difference of lactate became negative and a significant net release of lactate occurred after injection of the substances. These changes were associated with a significant decrease in oxygen uptake (table 8.3). In contrast with the elevated AV difference of lactate in group III (table 8.3), the AV differences of $K^+$, inorganic phosphate, pH and $p_{CO2}$ (table 8.4) were not significantly affected by the elevated arterial NEFA concentration.

### 8.3.4 In vitro effect of intralipid on hemoglobin (Hb) measurements

After administration of intralipid or intralipid-heparin, Hb concentrations increased consistently. If this is an artefact due to the intralipid administration errors are made in the calculations of oxygen uptake. To investigate whether this phenomenon was an artefact, various amounts of intralipid were added to freshly drawn arterial and venous blood and the Hb concentration was determined as described in section 3.5.3. The range of concentrations used in vitro was comparable with the blood concentrations of intralipid reached in the dog after injection of 50 ml 20% of this substance.

Both in vitro and in vivo, Hb values measured by the hemoxymeter increased with increasing concentrations of intralipid. $O_2$ saturation decreased proportionally so that $O_2$ content of the blood remained unchanged. This effect of intralipid is caused by absorption of light at the wavelengths used (Cane et

al, 1980).

## 8.4 Discussion

### 8.4.1 Artificial elevation of arterial NEFA

In the present study, the effect of increased arterial NEFA concentrations on MBF, myocardial metabolism and hemodynamics during ischemia was studied in anesthetized dogs. NEFA concentrations were elevated by intravenous injection of heparin, intralipid or intralipidheparin. The higher plasma NEFA concentrations reached after the administration of intralipid-heparin than after the injection of intralipid or heparin can be explained as follows. Intralipid causes primarily an increase of plasma triacylglycerol (Riemersma et al, 1977b) and only secondarily of plasma NEFA. The conversion into NEFA is stimulated by lipoprotein lipase which is released from the endothelium into the blood by heparin (Jansen, 1975; Riemersma et al, 1977a). It should be kept in mind that in group II and III no distinction can be made between the effect of high arterial NEFA concentrations alone or together with heparin and high arterial triacylglycerol concentrations.

### 8.4.2 Effect of elevated arterial NEFA concentrations on myocardial blood flow

The findings in our study indicate that highly elevated NEFA levels (0.66-1.23 mM) result in a decrease of total MBF, endo/epicardial blood flow ratio and oxygen uptake in the ischemic area. The decreased oxygen uptake is associated with a more pronounced net release of lactate from the ischemic myocardium, suggesting the augmentation of anaerobic glycolysis. These findings are consonant with the finding that there is a relation between these levels and infarct size reported by Opie et al (1977). The higher enzyme release from regional ischemic isolated rat hearts when perfused with a palmitic acid containing solution (De Leiris et al, 1975, 1978) could also be explained by a decreased ischemic blood flow. However, following coronary artery occlusion, Most et al (1974) did not observe an influence of intralipid-heparin administration on myocardial injury.

Although NEFA are known to have vasodilating properties in the coronary circulation (Huelsmann, 1976; Blass et al, 1978), it is unlikely that the decrease in MBF in the ischemic area results from a redistribution of blood flow in favor of non-ischemic areas. In our experimental set-up, after all,

poststenotic coronary artery pressure is kept constant. Increased serum viscosity, resulting in elevated resistance to blood flow, might be an explanation for the decrease in MBF in the ischemic myocardium. The administration of intralipid-heparin indeed resulted in increased serum viscosity as determined by capillary viscometry (unpublished results). Whether the change in serum viscosity is less pronounced after the administration of intralipid alone, which could explain the non-significant alteration in ischemic MBF under these circumstances, is not known. Neither do we have information about the influence of NEFA alone on serum or cellular viscosity.

It is unlikely that the differences in median values of ischemic MBF between group II and III before administration of the substances (table 8.2) influence the obtained results because no relation was found between these MBF values and changes in the determined variables after the administration of the substances within these groups.

The decrease in endo/epicardial blood flow ratio can possibly be explained by the finding that during stenosis arterioles are fully dilated in the subendocardium but only partially in the subepicardium (Gould et al, 1975). In this light increased viscosity of the blood will affect subepicardial blood flow only to a limited extent because the arterioles in this layer can still dilate to compensate for the increase in resistance caused by the elevated viscosity. This dilation may even be stimulated by the increased concentration of NEFA which are known to be coronary vasodilators (Huelsmann, 1976; Blass et al, 1978). The increased MBF in the non-ischemic area may also result from this property.

Whatever the reason of the decreased ischemic blood flow may be, this seems to be another deleterious effect of high circulating NEFA concentrations on the ischemic myocardium beside the presumed noxious effects of high intracellular NEFA, acyl-CoA and acylcarnitine concentrations in the myocytes (recently reviewed by Katz and Messineo, 1981; Van der Vusse, 1982) and the presence of proposed NEFA-driven "oxygen-wasting" processes in ischemic tissue (Vik-Mo and Mjøs, 1981).

8.4.3 Effect of elevated arterial NEFA concentration on myocardial
        metabolism

The increased net lactate release from the ischemic area is in agreement
with previous findings (Kjekhus and Mjøs, 1972) and indicates that acutely
elevated arterial NEFA concentrations do not inhibit anaerobic glycolysis in
the ischemic myocardium.

Lactate, potassium and inorganic phosphate are commonly used as markers
for ischemia. These markers are generally released simultaneously from the
myocardium within 20 min after onset of ischemia (Owen et al, 1970; Van der
Vusse et al, 1980). In the present study, a more severe degree of ischemia,
as indicated by the fall in MBF associated with increased net release of
lactate, does not result in an increased net release of potassium and inorga-
nic phosphate. One explanation for this discrepancy might be that the cells
are retaining potassium and inorganic phosphate. This theory is in agreement
with the finding that the AV differences of these substances after one hour
of ischemia do not significantly differ from those before ischemia, even when
a significant lactate release exists (Van der Vusse et al, 1980). Moreover,
the data referring to the net release of potassium and inorganic phosphate
during the first 60 min of ischemia (chapter 6 and 7) show that only approxi-
mately 10% of all potassium and approximately half of all liberated inorganic
phosphate is released into the venous blood draining the ischemic area. Ano-
ther possibility is that we missed the release of potassium and inorganic
phosphate because the majority of these markers is released within 10 min
after inducement of ischemia (Van der Vusse et al, 1980).

After administration of intralipid-heparin, net NEFA uptake tended to
increase while after intralipid administration net NEFA uptake became nega-
tive in 5 out of 8 dogs. The latter can be explained by an increased intra-
vascular lipolysis of serum triacylglycerol caused by an elevated concen-
tration of this substance. In this way, more NEFA are formed than can be
taken up by the myocardium and these NEFA are released into the venous efflu-
ence. This does not occur after administration of intralipid-heparin because
heparin releases the lipoprotein lipase from the endothelium (see above).

The increased arterial lactate concentrations after the administration of
intralipid as well as intralipid-heparin, reflect a systemic effect and might
result from release from skeletal muscles. The elevated arterial lactate
concentrations might be responsible for the not significantly changed net
NEFA uptake of the ischemic myocardium, in the presence of elevated arterial

NEFA levels since experiments in non-ischemic hearts have shown that the uptake of NEFA is diminished at elevated arterial lactate concentrations (Spitzer, 1974).

### 8.4.4 Effect of elevated NEFA concentration on hemodynamics

The increase in heart rate found after the administration of heparin as well as heparin and intralipid is probably caused by the former because heart rate did not change after the injection of intralipid alone. This is supported by the finding that intralipid administered to heparinized dogs had no significant effect on heart rate both in the non-ischemic (Mjøs, 1971a and b; Kjekhus and Mjøs, 1972; Most et al, 1973) and in the flow-restricted (Kjekhus and Mjøs, 1972) heart. It is unlikely that our experimental design is responsible for the increase in heart rate because in studies without intervention no significant changes in heart rate were found during 2 hours of stenosis (Van der Vusse et al, 1980, section 7).

The present findings indicate that elevated NEFA concentrations do not induce arrhythmias which is in accordance with data presented previously (Nelson, 1970a, b; Russo et al, 1970; Opie et al, 1971; De Leiris et al, 1975; Riemersma et al, 1977a; Riemersma and Oliver, 1979) but in disagreement with the findings of Oliver and Yates (1971) and Yamazaki (1978). This discrepancy is incompletely understood. A possible explanation might be that the plasma NEFA levels in our study were too low to induce arrhythmias (maximum 1.35 mM) because Oliver and Yates (1971) described their effects at plasma NEFA levels of 2.5 mM. On the other hand, Opie and co-investigators did not find an effect on arrhythmias at plasma NEFA levels of 6 mM (1971). Opie and Lubbe (1975) have stated in a good survey on the possible arrhythmogenic properties of free fatty acids that correct interpretation of the experimental findings can be influenced by species differences, experimental set-up (e.g. problems in the preparation of the NEFA:albumin complex, microelectrodes which could act as irritable focus) and concomitant raised catecholamine levels.

## 8.5 Conclusions

- A 4-fold elevation of arterial NEFA concentrations after 60 min of ischemia caused a decrease in total myocardial blood flow (MBF), endo/epicardial blood flow ratio and oxygen uptake in the ischemic myocardium. Such changes were not observed when arterial NEFA concentrations were only 1.5-2-fold elevated.
- The decrease in MBF mentioned above, was concomitted by a significant release of lactate but not of inorganic phosphate, potassium or $CO_2$.
- After elevation of arterial NEFA concentrations, no significant changes in glucose or NEFA uptake were observed.

9. SUMMARY AND CONCLUSIONS

The aim of the present study was to investigate the relation between fiber
shortening, blood flow and metabolism in the various layers of the left
ventricular wall during two hours of ischemia. Special attention is paid to
the cessation of fiber shortening and the accumulation of non-esterified
fatty acids (NEFA) in ischemic myocardium. Also the effect of artificially
elevated of arterial NEFA concentrations on the ischemic myocardium has been
studied. The experiments were performed on anesthetized open-chest dogs. Myo-
cardial blood flow (MBF) and metabolism were determined in the inner (suben-
docardial), middle and outer (subepicardial) layers of the left ventricular
wall, whereas fiber shortening was assessed in the inner and outer layers.
Fiber shortening was estimated from the epicardial surface deformation (Arts
and Reneman, 1980). MBF was determined with the radioactive microsphere
method (reviewed by Heymann et al, 1977). Myocardial metabolism was assessed
by determination of the contents of ATP, creatine phosphate, glycogen, NEFA,
triacylglycerol and phosphoglycerides in tissue samples from the myocardial
wall, and of myocardial uptake from the blood of oxygen, glucose, lactate,
NEFA, triacylglycerol, inorganic phosphate and potassium. Systemic hemodyna-
mic variables were measured as usual. Cardiac output was measured by thermo-
dilution according to Snoeckx et al (1976).

Ischemia was induced by partial or complete occlusion of the left anterior
interventricular coronary artery (LAICA) by inflating a cuff around this ar-
tery. The degree of partial occlusion (=stenosis) was estimated from the mean
coronary artery pressure ($p_{cor}$) distal to the stenosis. An appropriate degree
of stenosis was obtained by careful manual inflation of the cuff until a mean
$p_{cor}$ of 3.3 kPa (25 mmHg) was reached. Mean $p_{cor}$ was kept constant during the
experimental period with a servosystem controlled by this pressure.

Before studying the effects of ischemia, the experimental model was evalu-
ated during 2 hours without intervention (chapter 4). During this period, MBF
as well as the hemodynamical, regional mechanical and metabolic variables
were stable (i.e. did not change significantly). However, serial tissue
sampling during the experiments caused significant decreases in epicardial
deformation and MBF. Therefore, it was decided to terminate each experiment
after two biopsies had been taken, one from the ischemic (LAICA) and one from
the non-ischemic (LCCA) area. Changes due to ischemia can be evaluated by
comparing the content of a substance in the LAICA biopsy with that in the

LCCA biopsy of the same experiment since during normal perfusion, the contents of the various substances were similar in both regions.

In chapter 4, it has also been shown that MBF in the outer layer of the left ventricular free wall is inhomogeneously distributed. MBF in these layers was higher near the apex than near the base and lower in the posterior than in the anterior region of the free wall. The cause of these differences is incompletely understood.

In chapter 5, the prediction of fiber shortening in the inner layers from epicardial deformation was validated. Epicardial minimal $(e_{min})$ and maximal shortening $(e_{max})$ were calculated from the measured epicardial shortening in circumferential and base-to-apex direction and the shear angle during the ejection phase (section 3.3). According to a mathematical model of the left ventricle (Arts, 1978, 1980, 1982a,b), the direction of $e_{max}$ was approximately equal to the fiber direction in the outer layers and the direction of $e_{min}$ was close (within 0.3 rad) to the fiber direction in the inner layers. The relation between $e_{min}$ and shortening in the inner layers in the direction of $e_{min}$ (called $e_{endo}$) was subject of a study where deformation was assessed simultaneously at the epicardium and in the inner layers by measurement of mutual movement and angulation of three needles pierced into the myocardial wall. During normoxia and ischemia, $e_{min}$ was found to be clearly related to fiber shortening in the inner layers, so that information about this shortening could be obtained from measurements at the epicardium.

After onset of ischemia $e_{min}$ started to decrease 2 s (occlusion, chapter 5) and 30 s (stenosis, chapter 6) before a significant change in $e_{max}$ could be detected. $e_{min}$ transiently reached negative values (i.e. lengthening of the fibers), which was more pronounced after occlusion than after stenosis. However, $e_{max}$ gradually decreased to about 20% of the pre-ischemic values within the first min and the median did not reach negative values. These results show that after onset of acute ischemia fiber shortening is earlier and more severely affected in the inner than in the outer layers. Transmural differences in shortening are pronounced during the first half min but relatively small after one min of ischemia.

In chapter 6, the relation between this early cessation of shortening after stenosis of a coronary artery and the changes in MBF and metabolism was studied. Stenosis caused a decrease in MBF of 68% and 81% in the inner layers and of 36% and 54% in the outer layers after 1 and 5 min of ischemia, respectively. Within 5 min of ischemia, no significant changes could be detec-

ted in myocardial ATP content but creatine phosphate content in the inner layers had halved within one min of ischemia and further decreased gradually in all layers during the next 4 min. Significant release of inorganic phosphate into the venous blood draining the ischemic area, within half a minute of ischemia also indicated the early breakdown of energy-rich phosphates. Glycogen contents in the various layers were hardly affected within 5 min of ischemia. These results were compared with the current hypotheses on the cause of the early cessation of contractile activity as discussed in section 2.1.2. Based on the results of the present study the early cessation of shortening might be explained by the decrease in free energy provided by ATP hydrolysis. A major inhibition of shortening by local depletion of glycogen or accumulation of inorganic phosphate per se seems unlikely.

Because of the higher oxygen delivery (higher blood flow and probably small differences in $O_2$ extraction) to the outer than to the inner layers and because of the relatively small differences in the changes in fiber shortening during ischemia, it is suggested that the outer layers are impeded to shorten by the inner layers and are forced to bear stress which cannot be built up by the inner layers.

During the first 5 min of ischemia, the lactate release from the affected myocardium could only partially be explained by the uptake of glucose. An early, but hardly measurable glycogenolysis might be involved.

In chapter 7, the time course of blood flow, fiber shortening and metabolism during two hours of ischemia was studied. At the constant low perfusion pressure (see above), MBF to the whole ischemic area did not change significantly between 10 and 120 min of ischemia. In contrast, the relative underperfusion of the inner layers became worse at longer duration of ischemia. The tissue contents of ATP, creatine phosphate and glycogen after 10, 60, and 120 min of ischemia were diminished, the lowest values being reached in the inner layers. ATP content in the inner layers gradually declined to 28% of the normoxic value after 120 min of ischemia. In the inner layers, the creatine phosphate content after 60 min was about 25% of the normoxic value, but increased to about 50% of the normoxic value after 120 min. Glycogen content in the inner layers gradually decreased to 38% of the normoxic content after 120 of ischemia. The already severely affected regional myocardial fiber shortening after 10 min of ischemia, subsequently deteriorated during the next 110 min; $e_{max}$ declining to a slightly more pronounced degree than $e_{min}$. These results show that transmural differences in metabolism invariably exist

between 10 and 120 min, the transmural gradient in blood flow even increases within this period, and the differences in shortening in the myocardial wall tend to decrease.

No significant increase in NEFA content was observed 10 min after onset of ischemia. In the outer layers NEFA content showed a biphasic pattern with a maximum increase (about 3-fold) after 60 min of ischemia, whereas in the inner layers NEFA content gradually increased to approximately 4 times the normoxic values after 120 min. In the outer layers an important part of this accumulation may be caused by lipolysis of endogenous triacylglycerol or fatty acids extracted from the blood. The relatively highest increase in linoleic and arachidonic acid in the inner layers suggests that lipolysis of phosphoglycerides is an important source of these NEFA. NEFA accumulation in the inner layers after 60 min and in all layers after 120 min, proved to be related to MBF and ATP content of the ischemic area. After 120 min of ischemia NEFA contents were found to be increased when MBF was lower than 0.3 $ml.min^{-1}.g^{-1}$ and ATP content was lower than $10\,\mu mol.g^{-1}$ dry weight.

From these data it has been hypothetized that the NEFA accumulation in ischemic myocardium is likely not the cause of impaired mechanical performance and metabolism as well as cell death.

In chapter 8 the effect of elevated arterial NEFA concentrations on MBF, myocardial metabolism and hemodynamics during ischemia was studied. NEFA concentrations were elevated by intravenous injection of heparin, intralipid or both substances (intralipid-heparin).

After elevation of NEFA concentrations by heparin or intralipid-heparin, heart rate gradually increased, while aortic pressure tended to decrease.

Slight elevation of arterial NEFA levels (up to 0.53 mM), had no significant effect on total MBF and uptake of glucose, NEFA, and oxygen or release of lactate in the ischemic myocardium. However, elevating arterial NEFA levels up to 0.81 mM (by intralipid-heparin), significantly decreased total MBF (16%), ratio of blood flow in the inner and outer layers (13%) and oxygen uptake (34%) in the ischemic myocardium, and resulted in release of lactate from this area. The release of potassium, inorganic phosphate and $H^+$ as well as plasma $CO_2$ concentration were not influenced. Neither was the uptake of glucose and NEFA.

Although the role of the simultaneously elevated arterial triacylglycerol levels can not be excluded, these findings suggest that elevated arterial NEFA concentrations can decrease MBF and augment lactate production. Glycoly-

sis in the ischemic myocardium is not inhibited.

In short the findings of the present study indicate that:
- fiber shortening in the various layers of the left ventricular wall can be
  estimated from myocardial deformation during the ejection phase as measured
  at the epicardial surface
- after onset of ischemia, cessation of fiber shortening starts in the inner
  layers and subsequently is seen in the outer layers
- the cessation of fiber shortening after inducement of ischemia can not be
  ascribed to depletion of ATP or creatine phosphate stores
- during ischemia of one min and longer, transmural differences in fiber
  shortening are small compared to differences in blood flow and metabolic
  variables, suggesting that fiber shortening in the outer layers seems to be
  impeded by the severely affected function of the inner layers
- accumulation of NEFA in ischemic myocardium could be detected at 60 and 120
  min, but not after 10 min of ischemia
- the changes in mechanical function and metabolism in the ischemic myocar-
  dium are not necessarily due to NEFA accumulation
- an important part of the accumulated NEFA, especially in the inner layers
  after 120 min of ischemia, is probably derived from endogenous phospho-
  glycerides
- after 120 min of ischemia, accumulation of NEFA is highest in the inner
  layers and lowest in the outer layers. The extent of accumulation does not
  depend on the myocardial layer itself but on the amount of MBF and the
  content of ATP in the ischemic myocardium: increased NEFA contents are
  observed when myocardial blood flow is lower than $0.3$ ml.min$^{-1}$.g$^{-1}$ and when
  the ATP content is lower than $10\mu$mol.g$^{-1}$ dry weight.
- artificial 4-fold elevation of arterial NEFA concentrations by administra-
  tion of intralipid-heparin causes a 16% decrease in ischemic myocardial
  blood flow.

## 10. SAMENVATTING

Het hart is een holle spier, die dienst doet als pomp om het bloed door de bloedvaten voort te stuwen. De rechter en de linker harthelft bestaan ieder uit een dunwandige boezem, waarin de aders uitmonden, die het bloed naar het hart terugvoeren en een sterk gespierde kamer die het bloed de slagaders inpompt. De rechter harthelft ontvangt het zuurstofarme bloed uit de aders van het lichaam en pompt het naar de longen (de kleine bloedsomloop). De linker harthelft ontvangt het zuurstofrijke bloed uit de longen en pompt het naar alle organen van het lichaam (de grote bloedsomloop).

Het hart verkrijgt energie voor het rondpompen van het bloed door glucose, melkzuur en vetten te verbranden met behulp van zuurstof. Deze zuurstof wordt aangevoerd door het bloed in de kransslagaders. Vernauwing of afsluiting van een kransslagader leidt tot zuurstofgebrek (=ischemie) van de hartspier. Het optreden van ischemie van de hartspier leidt binnen een minuut tot verminderde samentrekking van de spiervezels (dus verminderde pompfunctie) en binnen een tot twee uur tot celdood. Indien dit laatste optreedt bij patienten, spreekt men van een hartinfarct.

Tijdens de hartcyclus is de doorstroming van de kransslagaders aan sterke veranderingen onderhevig, vooral in de linker kamer. Om het bloed de slagaders van het lichaam in te pompen wordt door samentrekking van de kamers een hoge druk opgebouwd in de kamerholte, die zich ook voortplant in de wand van de kamers. Deze weefseldruk belemmert echter de doorbloeding van de spierwand. Deze belemmering is het sterkst in de binnenste lagen van de linker kamerwand, waar de weefseldruk het hoogst is. Onder normale omstandigheden wordt het tekort aan doorbloeding tijdens samentrekking gecompenseerd door een versterkte doorstroming wanneer de hartspier ontspannen is. Echter wanneer de normale doorstroming door de kransslagaders gehinderd wordt door een vernauwing van het vat, schiet deze compensatie te kort. Hierdoor treedt ischemie het snelst op in de binnenste lagen van de linker kamer. Bij patienten wordt een hartinfarct overwegend in de binnenste lagen gevonden en experimenteel is aangetoond dat bij belemmerde doorstroming van de kransslagaders niet alleen de doorbloeding maar ook de stofwisseling van de binnenste lagen het sterkst verstoord wordt door ischemie.

Weinig is nog bekend over de verschillen in spiervezelverkorting binnen de kamerwand omdat het technisch moeilijk is de spiervezelverkorting te meten in de binnenste lagen van de wand.

Verhoogde concentraties van onveresterde (=vrije) vetzuren, zowel in de cel als in het bloed, worden door verschillende onderzoekers als schadelijk beschouwd voor het ischemische hartspierweefsel.

In het onderzoek, beschreven in dit proefschrift, is de relatie bestudeerd tussen doorbloeding, spiervezelverkorting en stofwisseling in verschillende lagen van de ischemische linker kamerwand. Het onderzoek vond plaats in honden met open borstkas, onder volledige verdoving. Ischemie van een gedeelte (10-20%) van de linker kamerwand is veroorzaakt door vernauwing of afsluiting van de voorste afdalende tak van de linker kransslagader.

De belangrijkste conclusies uit dit onderzoek zijn:
- spiervezelverkorting in de verschillende lagen van de linker kamerwand kan bepaald worden uit de vormverandering van deze wand, zoals deze gemeten wordt aan het buitenoppervlak van de ventrikel (het epicard).
- na het begin van ischemie neemt de spiervezelverkorting in de binnenste lagen van de kamerwand het eerst en het sterkst af, gevolgd door een meer geleidelijke afname in spiervezelverkorting in de buitenste lagen.
- de afname van de spiervezelverkorting na het aanleggen van ischemie, kan niet toegeschreven worden aan depletie van ATP of creatine fosfaat voorraden.
- gedurende ischemie van een minuut en langer zijn de verschillen tussen de binnenste en buitenste lagen van de kamerwand wat betreft spiervezelverkorting relatief klein ten opzichte van de verschillen in doorbloeding en stofwisseling. Een mogelijke verklaring hiervoor is dat spiervezelverkorting in de buitenste lagen van de kamerwand belemmerd wordt door de ernstig aangedane functie van de binnenste lagen van deze wand.
- ophoping van onveresterde vetzuren in ischemisch hartspierweefsel kon niet aangetoond worden na 10, maar wel na 60 en 120 min ischemie.
- een belangrijke oorzaak van de ophoping van onveresterde vetzuren, vooral in de binnenste lagen na 120 min ischemie, is waarschijnlijk de afbraak van fosfoglyceriden (fosfolipiden), stoffen die voorkomen in membranen van de cel.
- na 120 min, ischemie is de ophoping van onveresterde vetzuren het grootst in de binnenste lagen en het kleinst in de buitenste lagen van de linker kamerwand. De grootte van deze vetzuurophoping is echter niet afhankelijk van de laag in de wand, maar van de mate van afname van de doorbloeding en het ATP-gehalte in het ischemische hartspierweefsel: een verhoogd gehalte

onveresterde vetzuren is aangetroffen als de doorbloeding lager is dan 0.3 ml.min$^{-1}$.g$^{-1}$ en als het ATP gehalte lager is dan 10 $\mu$mol.g$^{-1}$ drooggewicht.

- de veranderingen in mechanische functie en stofwisseling in de ischemische linker kamerwand zijn waarschijnlijk niet te wijten aan de ophoping van onveresterde vetzuren, zoals wel is voorgesteld door andere onderzoekers op grond van proeven met geisoleerde harten of enzymsystemen.

- een viervoudige kunstmatige verhoging van de concentratie onveresterde vetzuren in het bloed veroorzaakte een afname van 16% van de doorbloeding in het ischemische deel van de linker kamerwand.

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## 11. LITERATURE

Albert, N.R., M.A. Gordon: Myofibrillar adenosine triphosphatase activity in congestive heart failure.
Am J. Physiol. 202: 940-946, 1962.

Allard, J.R., R.L. Conhaim, G.J. Vlahakes, M.J. O'Neill, and J.I.E. Hoffman: Rapid-freezing transmural cardiac biopsy drill.
Am. J. Physiol. 240: H126-H133, 1981.

Allella, A., F.L. Williams, C. Bolene-Williams, and L.N. Katz: Interrelation between cardiac oxygen consumption and coronary blood flow.
Am. J. Physiol. 183: 570-582, 1955.

Allison, T.B., C.A. Ramey, and J.W. Holsinger: Transmural gradients of left ventricular tissue metabolites after circumflex coronary artery ligation in dogs.
J. Mol. Cell. Cardiol. 9: 837-852, 1977.

Allison, T.B., C.A. Ramey, and J.W. Holsinger Jr.: Effects on labile metabolites of temporal delay in freezing biopsy samples of dog myocardium in liquid nitrogen.
Cardiovasc. Res. 12: 162-166, 1978.

Andrieu, J.L., C. Vial, B. Font, D. Goldschmidt, M. Lievre, and G. Faucon: Myocardial biochemical modifications induced by theophylline with reference to its value as antianginal drug.
Arch. Int. Pharmacodyn. 237: 330-342, 1979.

Andrieu, J.L., M. Lievre, Q. Timour Chah, and G. Faucon: Effects of propranolol on the biochemical modifications induced by a beta-adrenergic drug in ischemic hearts.
Biochem. Pharmacol. 29: 2687-2690, 1980.

Apstein, C.S., F. Gravino, W.B. Hood Jr., J. Frazer, L. Hagopian, and P. Bissell: Limitations of lactate production as an index of myocardial ischemia.
Circulation 60: 877-888, 1979.

Armour, J.A. and W.C. Randall: Canine left ventricular intramyocardial pressures.
Am. J. Physiol. 220: 1833-1839, 1971.

Arts, M.G.J.: A mathematical model of the dynamics of the left ventricle and the coronary circulation.
Ph.D.-thesis, University of Limburg, Maastricht, The Netherlands, 1978.

Arts, M.G.J. and R.S. Reneman: Analysis of intramyocardial pressure (MP).
Bibl. Anat. 15: 103-107, 1977.

Arts, M.G.J. and R.S. Reneman: Measurement of deformation of canine epicardium in vivo during cardiac cycle.
Am. J. Physiol. 239: H432-H437, 1980.

Arts, T., R.S. Reneman, and P.C. Veenstra: A model of the mechanics of the left ventricle.
Ann. Biomed. Eng. 7: 299-318, 1979.

Arts, T., S. Meerbaum, R.S.Reneman, and E. Corday: Cardiac wall mechanics and torsion of the left ventricle during ejection.
Proc. of the 3rd Meeting of the European Society of Biomechanics, 1982 (in press).

Arts, T., P.C. Veenstra, and R.S.Reneman: Epicardial deformation and left ventricular wall mechanics during ejection in the dog.
Am. J. Physiol. 1982 (in press).

Bache, R.J., Ph.A. McHale, and J.C. Greenfield Jr.: Transmural myocardial perfusion during restricted coronary inflow in the awake dog.
Am. J. Physiol. 232: H645-H651, 1977.

Ball, R.M., R.J. Bache, F.R. Cobb, and J.C. Greenfield Jr.: Regional myocardial blood flow during graded treadmill exercise in the dog.
J. Clin. Invest. 55: 43-49, 1975.

Banka, V.S., M.M. Bodenheimer, and R.H. Helfant: Relation between progressive decreases in regional coronary perfusion and contractile abnormalities.
Am. J. Cardiol. 40: 200-205, 1977.

Barany, M., E. Gaetjens, K. Barany, and E Karp: Comparative studies of rabbit cardiac and skeletal myosins.
Arch. Biochem. Biophys. 106: 280-293, 1964.

Berger, H.J., B.L. Zaret, L. Speroff, L.S. Cohen, and S. Wolfson: Regional cardiac prostaglandin release during myocardial ischemia in anesthetized dogs.
Circ. Res. 38: 566-571, 1976.

Bergmeyer, H.U., E. Bernt, F Schmidt, and H Stork: D-glucose determination with hexokinase and glucose-6-phosphate dehydrogenase.
In: Methods of enzymatic analysis. Ed. H.U. Bergmeyer, Academic Press, New York, N.Y., 1974, pp 1196-1201.

Berne, R.: Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow.
Am. J. Physiol. 204: 317-322, 1963.

Berry, J.W., D.G. Chappell, and R.B. Barnes: Improved method of flame photometry.
Ind. Eng. Chem. Anal. Ed. 18: 19-24, 1946.

Bishop, S.P., F.C. White, and C.M. Bloor: Regional myocardial blood flow during acute myocardial infarction in the conscious dog.
Circ. Res. 38: 429-438, 1976.

Blass, K.-E., P. Mentz, and W. Foerster: Effects of unsaturated fatty acids on canine coronary flow.
Acta Biol. Med. Ger. 37: 765-767, 1978.

Boerth, R.C., J.W. Covell, S.C. Seagren, and P.E. Pool: High-energy phosphate concentrations in dog myocardium during stress.
Am. J. Physiol. 216: 1103-1106, 1969.

Borst, P., J.A. Loos, E.J. Christ, and E.C. Slater: Uncoupling activity of long-chain fatty acids.
Biochim. Biophys. Acta 62: 509-518, 1962.

Bouskela, E., and C.A. Wiederhelm: Microvascular myogenic reaction in the wing of the intact unanesthetized bat.
Am J. Physiol. 237: H59-H65, 1979.

Braasch, W., S. Gudbjarnason, P.S. Puri, K.G. Ravens, and R.G. Bing: Early changes in ener-

gy metabolism in the myocardium following acute coronary artery occlusion in anesthetized dogs.
Circ. Res. 23: 429-438, 1968.

Bricknell, O.L. and L.H. Opie: Effects of substrates on tissue metabolic changes in the isolated rat heart during underperfusion and on release of lactate dehydrogenase and arrhythmias during reperfusion.
Circ. Res. 43:102-115, 1978.

Bricknell, O.L., P.S. Daries, and L.H. Opie: A relationship between adenosine triphosphate, glycolysis and ischemic contracture in the isolated rat heart.
J. Mol. Cell. Cardiol. 13: 941-945, 1981.

Buckberg, G.D., J.C. Luck, D.B. Payne, J.I.E. Hoffman, J.P. Archie, and D.E. Fixler: Some sources of error in measuring regional blood flow with radioactive microspheres.
J. Appl. Physiol. 31: 598-604, 1971.

Buckberg, G.D., D.E. Fixler, J.P. Archie, and J.I.E. Hoffman: Experimental subendocardial ischemia in dogs with normal coronary arteries.
Circ. Res. 30: 67-81, 1972.

Bui-Mong-Hung, G. Jarry, and D Laurant: Determination of left ventricular heat production in working dog heart.
J. Physiol., Paris 77: 711-719.

Burns, J.W., J.W.Covell, R. Myers, and J. Ross: Comparison of directly measured left ventricular wall stress and stress calculated from geometric reference figures.
Circ. Res. 28: 611-621, 1971.

Burrows, M.E. and P.C. Johnson: Diameter, wall tension, and flow in mesenteric arterioles during autoregulation.
Am. J. Physiol. 241: H829-H837, 1981.

Cane, R.D., R.A. Harrison, B.A. Shapiro, and J. Kavanaugh: The spectrophotometric absorbance of intralipid.
Anesthesiology 53: 53-55, 1980.

Capurro, N.L., K.C. Marr, R. Amoudt, R.E. Goldstein, and S.E. Epstein: Aspirin-induced increase in collateral flow after acute coronary occlusion in dogs.
Circulation 59: 744-748, 1979.

Carlsson, E.: Experimental studies of ventricular mechanics in dogs using the tantalum-labeled heart.
Fed. Proc. 28: 1324-1329, 1969.

Christie, W.W.: The isolation of lipids from tissues.
In: Lipid analysis. Pergamon Press, Oxford (1973), pp. 30-41 (chapter 2).

Cobb, F.R., R.J. Bache, and J.C. Greenfield Jr.: Regional myocardial blood flow in awake dogs.
J.Clin. Invest 53: 1618-1625, 1974.

Cobbe, S.M. and P.A. Poole-Wilson: Tissue acidosis in myocardial hypoxia.
J. Mol. Cell, Cardiol. 12: 761-770, 1980.

Coraboeuf, E., E. Deroubaix, and J. Hoerter: Control of ionic permeabilities in normal and ischemic heart.
Circ. Res. 38 (suppl. 1): 92-97, 1976.

Cowan, J.C. and E.M. Vaughan Williams: The effects of various fatty acids on action potential shortening during sequential periods of ischemia and reperfusion.
J. Mol. Cell. Cardiol. 12: 347-369, 1980.

Crass, M.F., E.S. McGaskill, J.C. Ship, and V.K. Murthy: Metabolism of endogenous lipids in cardiac muscle: Effect of pressure development.
Am. J. Physiol. 220: 428-435, 1971.

Crass, M.F. and G.M. Pieper: Lipid and glycogen metabolism in the hypoxic heart: Effects of epinephrine.
Am. J. Physiol. 229: 885-889, 1975.

Crass, M.F. and P.R. Sterrett: Distribution of glycogen and lipids in the ischemic canine left ventricle: biochemical and light and electron microscopic correlates.
Recent Adv. Stud. Cardiac Struct. Metab. 10: 251-261, 1975

Crozatier, B., M. Ashraf, D. Franklin, J. Ross Jr., L. Nimmo, and D. McKown: Sarcomere length in experimental myocardial infarction: evidence for sarcomere overstretch in dyskinetic ventricular regions.
J. Mol. Cell. Biol. 9: 785-797, 1977.

Crozatier, B., D. Franklin, H. Tomoike, and J. Ross Jr.: Nitroglycerine in experimental myocardial infarction in the baboon. The effect of reducing the precharge and postcharge on regional myocardial function.
Arch. mal Coeur 71: 701-710, 1978.

Crystal, G.J., R.B. Boatwright, H.F. Downey, and F.A. Bashour: Shunting of microspheres across the canine coronary circulation.
Am. J. Physiol. 236: H7-H12, 1979.

Dagenais, G.R. and B. Jalbert: Effect of increased free fatty acids on myocardial oxygen extraction and angina threshold during atrial pacing.
Circulation 56: 315-319, 1977.

Danforth, W.H.: Activation of glycolytic pathway in muscle.
In: Control of energy metabolism. (eds.: B. Chance, R.W. Eastbrook, J.R. Williamson), Academic Press, 1965, p 287-297.

Deboer, L.W.V., J.S. Ingwall, R.A. Kloner, and E. Braunwald: Prolonged derangements of canine myocardial purine metabolism after a brief coronary artery occlusion not associated with anatomic evidence or necrosis.
Proc. Nat. Acad. Sci. US Biol. Sci. 77: 5471-5476, 1980.

Dhalla, N.S., J.C. Yates, D.A. Walz, V.A. McDonald, and R.E. Olson: Correlation between changes in the endogenous energy stores and myocardial function due to hypoxia in the isolated perfused rat heart.
Can. J. Physiol. Pharmacol. 50: 333-345, 1972.

Domenech, R.J., J.I.E. Hoffman, M.I.M. Noble,

K.B. Saunders, J.R. Henson, and S. Subijanto:
Total and regional coronary blood flow mea-
sured by radioactive microspheres in con-
scious and anesthetized dogs.
Circ. Res. 25: 581-596, 1969.

Domenech, R.J.: Total and regional coronary
blood flow during acute coronary occlusion in
anesthetized and conscious dogs.
Cardiovasc. Res. 8: 415-422, 1974.

Dowell, R.T.: Transmural citrate synthase and
lactate dehydrogenase levels in hypertrophied
rat left ventricle.
Proc. Soc. Exp. Biol. Med. 158: 599-603,
1978.

Downey, J.M.: Myocardial contractile force as
a function of coronary blood flow.
Am. J. Physiol. 230: 1-6, 1976.

Downey, J.M., and E.S. Kirk: Inhibition of
coronary blood flow by a vascular waterfall
mechanism.
Circ. Res. 36: 753-760, 1975.

Downey, H.F., F.A. Bashour, B. Jishi, and
P.E. Parker: Arteriovenous shunts in dilated
or reperfused canine coronary vasculature.
Microvasc. Res. 17: 22-27, 1979.

Drake, A.J., J.R. Haines, and M.I.M. Noble:
Preferential uptake of lactate by the normal
myocardium in dogs.
Cardiovasc. Res. 14: 65-72, 1980a.

Drake, A.J., D.E. Papadoyannis, R.G. Butcher,
J. Stubbs, and M.I.M. Noble: Inhibition of
glycolysis in the denervated dog heart.
Circ. Res. 47: 338-345, 1980b.

Driscoll, T.E., and R.W. Eckstein: Coronary
inflow and outflow response to coronary
artery occlusion.
Circ. Res. 20: 485, 1967.

Dunn, R.B. and D.M. Griggs Jr.: Transmural
gradients in ventricular tissue metabolites
produced by stopping coronary blood flow in
the dog.
Circ. Res. 37: 438-445, 1975.

Dunn, R.B., K.M. McDonough, and D.M. Griggs
Jr.: Cardioacceleration by atrial pacing and
transmural metabolite levels in the canine
left ventricle.
Proc. Soc. Exp. Biol. Med. 158: 230-242,
1978.

Edwards, C.H., J. Scott Rankin, P.A McHale, D
Ling, and R.W. Anderson: Effects of ischemia
on left ventricular regional function in the
conscious dog.
Am. J. Physiol. 240: H413-H420, 1981.

Elshuraydeh, K: Cardiac dimensions and intra-
mural deformations.
Ph. D. thesis, University of Utrecht, The
Netherlands, 1981.

Estes, E.H. , M.L. Entman, H.B. Dixon, and
D.B. Hackel: The vascular supply of the left
ventricular wall.
Am. Heart J. 71: 58-67, 1966.

Evans, L.: Starling's principles of human
physiology. J.A. Churchill, London (1936),

pp. 706.

Evans, J.R.: Importance of fatty acid in
myocardial metabolism.
Circ. Res. 14 (suppl. 2): 96-108, 1964.

Fan, F. -C., G.B. Schuessler, R.Y.Z. Chen,
and S. Chien: Determinations of blood flow
and shunting of 9-um and 15-um spheres in
regional beds.
Am. J. Physiol. 237: H25-H34, 1979.

Feigl, E.O.: Control of myocardial oxygen
tension by sympathetic coronary vasoconstric-
triction in the dog.
Circ. Res. 37: 88-95, 1975[a].

Feigl, E.O.: Reflex parasympathetic coronary
vasodilatation elicited from cardiac recep-
tors in the dog.
Circ. Res. 37: 175-182, 1975[b].

Feigl, E.O. and D.L. Fry: Intramural myocar-
dial shear during the cardiac cycle.
Circ. Res. 14: 536-540, 1964.

Feigl, E.O., G.A. Simon, and D.L. Fry: Auxo-
tonic and isometric cardiac force transdu-
cers.
J. Appl. Physiol. 23: 597-600, 1967.

Fenton, T.R., J.M. Cherry, and G.A. Klassen:
Transmural myocardial deformation in the
canine left ventricular wall.
Am. J. Physiol. 235, H523-H530, 1978.

Feuvray, D., J.A. Idell-Wenger, and J.R.
Neely: Effects of ischemia on rat myocardial
function and metabolism in diabetes.
Circ. Res. 44: 322-329, 1979.

Feuvray, D. and J. Plouet: Relationship
between structure and fatty acid metabolism
in mitochondria isolated from ischemic rat
hearts.
Circ. Res. 48: 740-747, 1981.

Fiske, C.H. and Y. Subbarow: The colorimetric
determination of phosphorus.
J.Biol. Chem. 66: 375-400, 1926.

Flameng, F., B. Winkler, B. Wuesten, and W.
Schaper: Minimum requirements for the mea-
surement of regional myocardial flow using
tracer microspheres.
Bibl. Anat. 15: 24-29, 1977.

Franklin, D.: The determination of myocardial
wall thickness by ultrasonic means: general
discussion.
In: Cardiovascular applications of ultra-
    sound. Ed. R.S. Reneman, North- Holland
    Publishing Company, Amsterdam, 1974, p
    454.

Franson, R.C., D.C. Pang, D.W. Towle, and
W.B. Weglicki: Phospholipase A activity of
highly enriched preparations of cardiac
sarcolemma from hamster and dog.
J. Mol. Cell. Cardiol. 10: 921-930, 1978.

Fung, Y.-Ch.: Stochastic flow in capillary
blood vessels.
Microvasc. Res. 5: 34-48, 1973.

Gallagher, K.P., T. Kumada, J.B. Reese, D.
McKown, and J. Ross Jr.: Correlation of

regional myocardial blood flow and function
during limited coronary inflow in dogs.
Circulation 57/58 (suppl. II): 56, 1978
(abstract).
Genain, C., P. Theroux, Ch. Thuillez, G.M.
Gourassa, and D.D. Waters: The interrela-
tionships between function and flow in the
subendocardial and subepicardial regions of
the left ventricle.
Circulation 59/60 (suppl. II): 28, 1979
(abstract).

Gertz, E.W., J.A. Wisneski, R. Neese, A.
Houser, R. Korte, and J.D. Bristow: Myocar-
dial lactate extraction. Multi-determined
metabolic function.
Circulation 61: 256-262, 1980.

Gevers, W.: Generation of protons by meta-
bolic processes in heart cells.
J.Moll. Cell. Cardiol. 9: 867-874, 1977.

Giacomini, K.M., S.E. Swezey, J.C. Giacomini,
and T.F. Blaschke: Administration of heparin
causes in vitro release of non-esterified
fatty acids in human plasma.
Life Sci. 27: 771-781, 1980.

Gibbs, C.L.: Cardiac energetics.
Physiol. Rev. 58: 174-255, 1978.

Giesen, J. and H. Kammermeier: Relationship
of phosphorylation potential and oxygen
consumption in isolated perfused rat hearts.
J. Mol.Cell. Cardiol. 12: 891-909, 1980.

Gloster, J. and P. Harris: Fatty acid binding
to cytoplasmic proteins of myocardium and red
and white skeletal muscle in the rat. A pos-
sible new role for myoglobin.
Biochem. Biophys. Res. Commun. 74: 506-513,
1977.

Goldfarb, R.D. and Th.M. Glenn: Beneficial
effects of prostaglandin $F_2$ alpha in experi-
mental myocardial infarction.
Am. J. Cardiol. 33: 139, 1974 (abstract).

Gould, K.L., K. Lipscomb, and C. Calvert:
Compensatory changes of the distal coronary
vascular bed during progressive coronary
constriction.
Circulation 51: 1085-1094, 1975.

Gregg, D.E.: The natural history of coronary
collateral development.
Circ. Res. 35: 335-344, 1974.

Griggs, D.M., V.V. Tchokoev, and C.C. Chen:
Transmural differences in ventricular tissue
substrate levels due to coronary constric-
tion.
Am. J. Physiol. 222: 705-709, 1972.

Grimm, A.F., H.L. Lin, and B.R. Grimm: Left
ventricular free wall and intraventricular
pressure-sarcomere length distributions.
Am. J. Physiol. 239: H101-H107, 1980.

Gudbjarnason, S., P. Mathes, and K.G. Ravens:
Functional compartmentation of ATP and crea-
tine phosphate in heart muscle.
J. Mol. Cell. Cardiol. 1: 325-339, 1970.

Gutmann, I., and A.W. Wahlefeld: L-lactate
determination with lactate dehydrogenase and
NAD.

In: Methods of enzymatic analysis. Ed. H.U.
Bergmeyer, Academic Press, New York,
N.Y., 1974, pp 1464-1468.

Guntheroth, W.G.: In vivo measurements of di-
mensions of veins with implications regarding
control of venous return.
IEEE Trans. Biomed. Eng. 16: 247-253, 1969.

Haider, B., S.S. Ahmed, C.B. Moschos, H.A.
Oldewurtel, and T.R. Regan: Myocardial func-
tion and coronary blood flow response to
acute ischemia in chronic canine diabetes.
Circ. Res. 40: 577-584, 1977.

Hales, J.R.S., R.B. King, and A.A. Fawcett:
Observations on the validity of using NEN-
Trac microspheres for measuring organ blood
flow.
Pfluegers Arch. 379: 295-296, 1979.

Hearse, D.J.: Oxygen deprivation and early
myocardial contractile failure. Reassessment
of the possible role of adenosine triphos-
phate.
Am. J. Cardiol. 44: 1115-1122, 1979.

Hearse, D.J. and D.A. Stewart: Functional
recovery of the myocardium after elective
cardiac arrest in the isolated rat heart.
The Lancet i: 192-194, 1974.

Hearse, D.J. and D.A. Chappell: A multiple
head tissue sampler drill for regional myo-
cardial studies.
J. Mol. Cell. Cardiol. 8: 803-809, 1976.

Hess, D.S. and R.J. Bache: Transmural right
ventricular myocardial blood flow during
systole in the awake dog.
Circ. Res. 45: 88-94, 1979.

Hewitt, R.L., D.M. Lolley, G.A. Adrouny, and
T. Drapanas: Protective effect of glycogen
and glucose on the anoxic arrested heart.
Surgery 75: 1-10, 1974.

Heymann, M.A., B.D. Payne, J.I.E. Hoffman,
and A.M. Rudolph: Blood flow measurements
with radionuclide-labeled particles.
Prog. Cardiovasc. Dis. 20: 55-79, 1977.

Heyndrickx, G.F., R.W. Millard, R.J. MacRit-
chie, P.R. Maroko, and S.F. Vatner: Regional
myocardial functional and electrophysiologi-
cal alterations after brief coronary artery
occlusion in conscious dogs.
J. Clin. Invest. 56: 978-985, 1975.

Higgins, T.J.C., P.J. Bailey, and D. Allsopp:
The influence of ATP depletion on the action
of phospholipase C on cardiac myocyte mem-
brane phospholipids.
J. Mol. Cell. Cardiol. 13: 1027-1030, 1981.

Higuchi, M. E. Tomomatsu, and K. Nishi:
Transmural distribution of myocardial high
energy phosphates and lactate in relation to
the epicardial ECG in the underperfused
canine heart.
Pfluegers Arch. 391: 101-104, 1981.

Hinds, J.E., E.W. Hawthorne, C.B. Mullins,
and J.H. Mitchel: Instantaneous changes in
the ventricular lengths occurring in dogs
during the cardiac cycle.
Federation Proc. 28: 1351-1357, 1969.

Hirzel, H.O., G.R. Nelson, E.H. Sonnenblick, and E.S. Kirk: Redistribution of collateral blood flow from necrotic to surviving myocardium following coronary occlusion in the dog. Circ. Res. 39: 214-222, 1976.

Hoffbrand, B.I. and R.P. Forsyth: Validity studies of the radioactive microsphere method for the study of the distribution of cardiac output, organ blood flow, and resistance in the conscious rhesus monkey. Cardiovasc. Res. 3: 326-342, 1969.

Hoffman, J.I.E.: Determinants and prediction of transmural myocardial perfusion. Circulation 58: 381-391, 1978.

Hoffman, J.I.E.: The effect of intramyocardial forces on the distribution of intramyocardial blood flow. J. Biomed. Eng. 1: 33-40., 1979.

Holtz, J., W.A. Grunewald, R. Manz, W. van Restorff, and E. Bassenge: Intracapillary hemoglobine oxygen saturation and oxygen consumption in different layers of the left ventricular myocardium. Pfluegers Arch. 370: 253-258, 1977.

Hood Jr., W.B., V.H. Covelli, W.H. Abelmann, and J.C. Norman: Persistence of contractile behavior in acutely ischemic myocardium. Cardiovasc. Res. 3: 249-260, 1969.

Hort, W.: Makroskopische und mikrometrische Untersuchungen am Myokard verschieden stark gefuellter linker Kammern. Arch. Path. Anat. Physiol. 333: 523, 1960.

Hough, F.S. and W. Gevers: Catecholamine release as mediator of intracellular enzyme activation in ischemic perfused rat hearts. S. Afr. Med. J. 49: 538-543, 1975.

Huisman, R.M., G. Elzinga, N. Westerhof, and P. Sipkema: Measurement of left ventricular wall stress. Cardiovasc. Res. 14: 142-153, 1980.

Huelsmann, W.C.: Coronary vasodilatation by fatty acids. Basic Res. Cardiol. 71: 179-182, 1976.

Hunneman, D.H., C. Schweickhardt, M.M. Gebhard, C.J. Preusse, and H.J. Bretschneider: Intramyocardial FFA concentration after treatment with verapamil. Pfluegers Arch. suppl. 391: R12, 1981 (abstract).

Ichihara, K. and Y. Abiko: Differences between endocardial and epicardial utilization of glycogen in the ischemic heart. Am. J. Physiol. 229: 1585-1589, 1975.

Ichihara, K. and Y. Abiko: Inhibition of endo- and epicardial glycogenolysis by propranolol in ischemic hearts. Am. J. Physiol. 232: H349-H353, 1977.

Ichihara, K., M. Ichihara, and Y. Abiko: Possible role of beta-adrenergic receptors in myocardial metabolic response to ischemia. In: Perspectives in cardiovascular research, vol 3. Ischemic myocardium and antianginal drugs (eds.: M.M. Winbury, Y. Abiko). Raven Press, New York, 1979, pp. 17-28.

Idell-Wenger, J.A., L.W. Grotyohan, and J.R. Neely: Coenzyme A and carnitine distribution in normal and ischemic hearts. J. Biol. Chem. 253: 4310-4319, 1978.

Imai, Sh., Y. Katano, N. Shimamoto, and K. Sakai: Energy metabolism in ischemic myocardium as studied in the isolated perfused guinea pig heart. In: Perspectives in cardiovascular research. Ischemic myocardium and antianginal drugs (eds.: M.M. Winbury, Y. Abiko). Raven Press, New York (1979), pp. 185-199 (vol. 3).

Ingels, N.B., G.T. Daughters, and S.R. Davies: Stereo photogrammetric studies on the dynamic geometry of the canine left ventricular epicardium. J Biomech. 4: 541-550, 1971.

Irisawa, H., A.P. Greer, and R.F. Rushmer: Changes in the dimensions of the vena cava. Am. J. Physiol. 196: 741-744, 1959.

Jageneau, A.H.M., W. van Gerven, R. Kruger, H. van Belle, and R.S. Reneman: An improved animal model for studying the effect of drugs on myocardial metabolism during ischemia. Recent Adv. Stud. Cardiac Struct. Metab. 10: 331-341, 1975.

Jansen, H.: Lipolytic activities in postheparin serum. Ph.D-thesis, Medical Faculty, Rotterdam, The Netherlands (1975).

Jedeikin, L.A.: Regional distribution of glycogen and phosphorylase in the ventricles of the heart. Circulation 45: 202-210, 1964.

Jennings, R.B. and C.E. Ganote: Mitochondrial structure and function in acute myocardial ischemic injury. Circ. Res. 38 (suppl. 1): 80-89, 1976.

Jennings, R.B., H.K. Hawkins, J.E. Lowe, M.L. Hill, S. Klotman, and K.A. Reimer: Relation between high energy phosphate and lethal injury in myocardial ischemia in the dog. Am. J. Pathol. 92: 187-215, 1978.

Jennings, R.B., K.A. Reimer, M.L. Hill, and S.E. Mayer: Total ischemia in dog hearts in vitro. 1. Comparison of high-energy phosphate production, utilization and depletion and of adenine nucleotide catabolism in total ischemia in vitro vs. severe ischemia in vivo. Circ. Res. 49: 892-900, 1981.

Jesmok, G.J., D.C. Warltier, G.J. Gross, and H.F. Hardman: Transmural triglycerides in acute myocardial ischemia. Cardiovasc. Res. 12: 659-666, 1978.

Jones, C.E., J.X. Thomas, J.C. Parker, and R.E. Parker: Acute changes in high energy phosphates, nucleotide derivatives, and contractile force in ischemic and non-ischemic canine myocardium following coronary occlusion. Cardiovasc. Res. 10: 275-282, 1976.

Jong, J.W. de, and S. Goldstein: Changes in coronary venous inosine concentration and myocardial wall thickening during regional

ischemia in the pig.
Circ. Res. 35: 111-116, 1974.

Jong, J.W. de, R.G. Merin, and H.C. Scham-hardt: Influence of different anesthetics on myocardial performance and metabolism.
J. Mol. Cell. Cardiol. 9 (suppl.): 60, 1977a (abstract).

Jong, J.W. de, P.D. Verdouw, and W.J. Remme: Myocardial nucleoside and carbohydrate meta-bolism and hemodynamics during partial occlu-sion and reperfusion of pig coronary artery.
J. Mol. Cell. Cardiol. 9: 297-312, 1977b.

Jonge, de: Inleiding tot de medische statis-tiek. Deel II, p 653. Ned. Inst. voor Prev. Geneesk., Leiden, 1964.

Kako, J., and R.J. Bing: Contractility of actin myosin bands prepared from normal and failing human hearts.
J. Clin. Invest. 37: 465-470, 1958.

Kammermeier, H., W. Krautzberger, and B. Kammermeier: Myocardial high energy phos-phates as influenced by catecholamines and pressure load.
Pfluegers Arch. 349: 325-335, 1974.

Kammermeier, H., E. Juengling, and P. Schmidt: Hypoxic failure: energy deficiency or E-M-decoupling=
J. Mol. Cell. Cardiol. 13 (suppl. 1): 42, 1981 (abstract).

Katz, A.M.: Effects of interrupted coronary flow upon myocardial metabolism and contrac-tility.
Prog. Cardiovasc. Dis. 10: 450-465, 1968.

Katz, A.M., and J.B. Maxwell: Actin from heart muscle: sulphydryl groups.
Circ. Res. 14: 345-350, 1964.

Katz, A.M. and H.H. Hecht: The early "pump" failure of the ischemic heart.
Am. J. Med. 47: 1497-1502, 1969.

Katz, A.M. and F.C. Messineo: Lipid-membrane interactions and the pathogenesis of ischemic damage in the myocardium.
Circ. Res. 48: 1-17, 1981.

Kjekshus, J.K. and O.D. Mjøs: Effect of free fatty acids on myocardial function and meta-bolism in ischemic dog heart.
J. Clin. Invest. 51: 1767-1776, 1972.

Klein, H.H., J. Schaper, St. Puschmann, Ch. Nienaber, H. Kreuzer, and W. Schaper: Loss of canine myocardial nicotinamide adenine dinu-cleotides determines the transition from reversible to irreversible ischemic damage of myocardial cells.
Basic. Res. Cardiol. 76: 612-621, 1981.

Kleinert, H.D. and H.R. Weiss: Blood flow and high-energy phosphates in microregions of left ventricular subendocardium.
Am. J. Physiol. 249: H804-H810, 1981.

Klocke, F.J., E. Braunwald, J Ross: Oxygen cost of electrical activation of the heart.
Circ. Res. 18: 357-365, 1966.

Kramer, J.K.G. and H.W. Hulan: The effect of dietary erucic acid on cardiac triglycerides and free fatty acid levels in rats.
Lipids 13: 438-444, 1978.

Krause, E.G., A. Ziegelhoffer, M. Fedelsova, J. Styk, S. Kostolanski, I. Gabauer, I. Blasig, and A. Wollenberger: Myocardial cyclic nucleotide levels following coronary artery ligation.
Adv. Cardiol. 25: 119-130, 1977.

Kuebler, W. and P.G. Spieckermann: Regulation of glycolysis in the ischemic and the anoxic myocardium.
J. Mol. Cell. Cardiol. 1: 351-377, 1970.

Kuebler, W. and A.M. Katz: Mechanism of early pump failure of ischemic heart. Possible role of adenosine triphosphate depletion and inorganic phosphate accumulation.
Am. J. Cardiol. 40: 467-471, 1977.

Kurien, V.A. and Oliver, M.F.: Serum free fatty acids after acute myocardial infarction and cerebral vascular occlusion.
The Lancet ii: 122-127, 1966.

Lab, M.J. and K.V. Woollard: Monophasic action potentials, electrocardiograms and mechanical performance in normal and ischemic epicardial segments of the pig ventricle in situ.
Cardiovasc. Res. 12: 555-566, 1978.

Lamprecht, W., and I. Trautschold: Determina-tion of adenosine triphosphate.
In: Methods of enzymatic analysis. H.U. Bergmeyer, Ed., Academic Press, New York, N.Y., 1974, pp. 2101-2110.

Lamprecht, W., P. Stein, F. Heinz, and H. Weisser: Determination of creatine phosphate.
In: Methods of enzymatic analysis. H.U. Bergmeyer, Ed., Academic Press, New York, N.Y., 1974, pp. 1777-1781.

Lea, M.A. and G. Weber: Role of enzymes in homeostasis. VIII. Inhibition of the activity of glycolytic enzymes by free fatty acids.
J. Biol. Chem. 243: 1096-1102, 1968.

Leiris, J. de, L.H. Opie, and W.F. Lubbe: Effect of free fatty acid and enzyme release in experimental glucose on myocardial infarc-tion.
Nature 253: 746-747, 1975.

Leiris, J. de, and L.H. Opei: Effect of substrates and of coronary artery ligation on mechanical performance and on release of lac-tate dehydrogenase and creatine phosphokinase in isolated working rat hearts.
Cardiovasc. Res. 12: 585-597, 1978.

Liedtke, A.J.: Alterations of carbohydrate and lipid metabolism in the acutely ischemic heart.
Prog. Cardiovasc. Dis. 23: 321-336, 1981.

Liedtke, A.J., S. Nellis, and J.R. Neely: Effects of excess free fatty acids on mecha-nical and metabolic function in normal and ischemic myocardium in swine.
Circ. Res. 43: 652-62, 1978.

Liedtke, A.J., H.C. Hughes, and J.R. Neely: An experimental model for studying myocardial

ischemia. Correlation of hemodynamic perfor-mance and metabolism in the working swine heart.
J. Thorac. Cardiovasc. Surg. 69: 203-211, 1975.

Lochner, A., J.C.N. Kotzé, A.J.S. Benade, and W. Gevers: Mitochondrial oxidative phosphory-lation in low-flow hypoxia: Role of free fatty acids.
J. Mol. Cell. Cardiol. 10: 857-875, 1978.

Lundsgaard-Hansen, P., C. Meyer, H. Riedwyl, E. Ehrengruber, E. Streit, and U. Schreiber: Transmural gradients of glycolytic enzyme activities in left ventricular myocardium. I. The normal state.
Pfluegers Arch. 297: 89-106, 1967.

Marcus, M.L., R.E. Kerber, J. Ehrhardt, and F.M. Abboud: Three-dimensional geometry of acutely ischemic myocardium.
Circulation 52: 254-263, 1975.

Marcus, M.L., R.E. Kerber, J. Ehrhardt, F.M. Abboud, M. Schrader, D. Panther, and O. Lim: Effects of time on volume and distribution of coronary collateral flow.
Am. J. Physiol. 230: 279-285, 1976.

Marsboom, R.A., D. Verstraete, D. Thienpont, and D. Mattheeuws: The use of halo-anisone and fentanyl for neuroleptanalgesia in dogs.
Br. Vet. J. 120: 466-468, 1964.

Marzilli, M., H.N. Sabbah, and P.D. Stein: Supply-demand balance of subendocardial muscle. Estimation from intramyocardial pres-sure.
J. Thorac. Cardiovasc. Surg. 79: 803-809, 1980.

Marzilli, M., P. Camici, H. Sabbah, P.D. Stein, and F. Ursini: Indirect evidence of regional differences of oxygen need within the left ventricle.
J. Mol. Cell. Cardiol. 13 (suppl. I): 55, 1981 (abstract).

McHale, P.A., and J.C. Greenfield: Evaluation of several geometric models for estimation of left ventricular circumferential wall stress.
Circ. Res. 33: 303-312, 1973.

McKeever, W.P., D.E. Gregg, and P.C. Canney: Oxygen uptake of the nonworking left ventri-cle.
Circ. Res. 6: 612-623, 1958.

Meduski, J.W., B.C. Abbott, and J.D. Meduski: Physiological aspects of the differences in the ability of mammalian cytomembranes to interact with molecular oxygen (abstract).
Fed. Proc. 36: 567, 1977.

Meer, J.J. van der, and R.S. Reneman: An improved technique to induce a standardized functional stenosis of a coronary artery.
Eur. Surg. Res. 4: 407-418, 1972.

Meer, J.J. van der, A.H.M. Jageneau, G. Elzinga, R. van Grondell, and R.S. Reneman: Changes in myocardial wall thickness (MWT) in open-chest dogs.
Pfluegers Arch. 340: 35-50, 1973.

Mishkin, S., L. Stein, Z. Gatmaitan, and I.M.

Arias: The binding of fatty acids to cyto-plasmic proteins: binding to Z-protein in liver and other tissues of the rat.
Biochem. Biophys. Res. Commun. 47: 997-1003, 1972.

Mjøs, O.D.: Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs.
J. Clin. Invest. 50: 1386-1389, 1971[a].

Mjøs, O.D.: Free fatty acids and oxygen consumption in dogs.
Scand. J. Clin. Lab. Invest. 28: 121-125, 1971[b].

Mohrman, D.E. and E.A. Feigl: Competition between sympathetic vasoconstriction and metabolic vasodilatation in canine coronary circulation.
Circ. Res. 42: 79-86, 1978.

Morgan, H.E., P.J. Randle, and D.M. Regen: Regulation of glucose uptake by muscle. 3. The effects of insulin, anoxia, salicate and 2:4-dinitrophenol on membrane transport and intracellular phosphorylation of glucose in the isolated heart.
Biochem. J. 73: 573-579, 1959.

Most, A.S., R.J. Capone, P. Szydlik, C.A. Bruno and T.S. DeVona: Failure of free fatty acids to influence the degree of myocardial injury following acute coronary artery occlu-sion in pigs.
Cardiology 59: 201-212, 1974.

Nakamura, M., H. Matsuguchi, A. Mitsutake, Y. Kikuchi, A. Takeshita, and O. Nakagaki: Effect of graded coronary stenosis on myocar-dial blood flow and left ventricular wall motion.
Cardiology 72: 479-491, 1977.

Nayler, W.G.: The role of calcium in the ischemic myocardium.
Am. J. Pathol. 102: 262-270, 1981.

Nayler, W.G., P.A. Poole-Wilson, and A. Williams: Hypoxia and calcium.
J. Mol. Cell. Cardiol. 11: 683-707, 1979.

Neely, J.R., and H.E. Morgan: Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle.
Ann. Rev. Physiol. 36: 413-459, 1974.

Neely, J.R., J.T. Whitmer, and M.J. Rovetto: Effects of coronary blood flow on glycolytic flux and intracellular pH in isolated rat hearts.
Circ. Res. 37: 733-741, 1975.

Nelson, P.G.: Free fatty acids and cardiac arrhythmias.
The lancet i: 783, 1970[a].

Nelson, P.G.: Effect of heparin on serum free fatty acids, plasma catecholamines, and the incidence of arrhythmias following acute myocardial infarction.
Br. Med. J.: 3: 735-737, 1970[b].

Obeid, A., H. Smulyan, R. Gilbert, and R.H. Eich: Regional metabolic changes in the myocardium following coronary artery ligation in dogs.

Am. Heart J. 83: 189-196, 1972.

Ockner, R.K., J.A. Manning, R.B. Poppenhausen, and W.K.L. Ho: A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues.
Science 177: 56-58, 1972.

Oliver, M.F., V.A. Kurien, and T.W. Greenwood: Relation between serum free fatty acids and arrhythmias and death after acute myocardial infarction.
The Lancet 1: 710-715, 1968.

Oliver, M.F. and P.A. Yates: Induction of ventricular arrhythmias by elevation of arterial free fatty acids in experimental myocardial infarction.
Cardiology 56: 359-364, 1971.

Opie, L.H.: Metabolism of the heart in health and disease, Part I.
Am. Heart J. 76: 685-698, 1968.

Opie, L.H.: Substrate utilization and glycolysis in the heart.
Cardiology 56: 2-21, 1971/1972.

Opie, L.H.: Effects of regional ischemia on metabolism of glucose and fatty acids.
Circulation 38: Suppl.I: I-52-I-68, 1976.

Opie, L.H. and K.R.L. Mansford: The value of lactate and pyruvate measurements in the assessment of the redox state of free nicotinamide adenine dinucleotide in the cytoplasma of perfused rat heart.
Eur. J. Clin. Invest. 1: 295-306, 1971.

Opie, L.H., P.Thomas, P. Owen, R.M. Norris, A.J. Holland, and S. van Noorden: Failure of high concentrations of circulating free fatty acids to provoke arrhythmias in experimental myocardial infarction.
The Lancet 1: 818-822, 1971.

Opie, L.H., M. Thomas, P. Owen and G. Shulman: Increased coronary venous inorganic phosphate concentrations during experimental myocardial ischemia.
Am. J. Cardiol. 30: 503-513, 1972.

Opie, L.H., P. Owen, and R.A. Riemersma: Relative rates of oxidation of glucose and free fatty acids by ischemic and nonischemic myocardium after coronary artery ligation in the dog.
Eur. J. Clin. Invest. 3: 419-435, 1973.

Opie, L.H., K. Bruyneel, and P. Owen: Effects of glucose, insulin and potassium infusion on tissue metabolic changes within the first hour of myocardial infarction in the baboon.
Circulation 52: 49-57, 1975.

Opie, L.H., and W.F. Lubbe: Are free fatty acids arrhythmogenic=
J. Mol. Cell. Cardiol. 7: 155-159, 1975.

Opie, L.H. and P. Owen: Effect of glucose-insulin-potassium infusions on arterio-venous differences of glucose and of free fatty acids on tissue metabolic changes in dogs with developing myocardial infarction.
Am. J. Cardiol. 38: 310-321, 1976.

Opie, L.H., P. Owen, and W. Lubbe: Estimated

glycolytic flux in infarcting heart.
Recent Adv. Stud. Cardiac Struct. Metab. 7: 249-255, 1976.

Owen, P., M. Thomas, V. Young, and L.H. Opie: Comparison between metabolic changes in local venous and coronary sinus blood after acute experimental coronary arterial occlusion.
Am. J. Cardiol. 25: 562-570, 1970.

Pitts, B.J.R., C.A. Tate, W.B. Vanwinkle, M.J. Wood, and M.L. Entman: Palmitylcarnitine inhibition of calcium pump in cardiac sarcoplasmic reticulum. Possible role in myocardial ischemia.
Life Sci. 23: 391-403, 1978.

Phibbs, R.H. and L. Dong: Nonuniform distribution of microspheres in blood flowing through a medium-size artery.
Can. J. Physiol. Pharmacol. 48: 415-421, 1970.

Poole-Wilson, P.A.: Is early decline of cardiac function in ischemia due to carbon dioxide retention=
Lancet 2: 1285-1287, 1975

Poole-Wilson, P.A. and G.A. Langer: The effect of acidosis and manganese on calcium exchange in the myocardium of the rabbit.
J. Physiol. 265: 20-21, 1976.

Pressman, B.C. and H.A. Lardy: Effect of active agents on the latent ATPase of mitochondria.
Biochim. Biophys. Acta 21: 458-466, 1956.

Prokop, E.K., H.W. Strauss, J. Shaw, B. Pitt, and H.N. Wagner Jr.: Comparison of regional myocardial perfusion determined by ionic potassium-43 to that determined by microspheres.
Circulation 50: 978-984, 1974.

Ramadoss, C.S., K. Uyeda, and J.M. Johnston: Studies on the fatty acid inactivation of phosphofructokinase.
J.Biol. Chem. 251: 98-107, 1976.

Randle, P.J., E.A. Newsholme, and P.B. Garland: Regulation of glucose uptake by muscle. 8. Effects of fatty acids, keton bodies and pyruvate, and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscle.
Biochem. J. 93: 652-665, 1964.

Rau, E.E., K.I. Shine, and G.A. Langer: Potassium exchange and mechanical performance in anoxic mammalian myocardium.
Am. J. Physiol. 232: H85-H94, 1977.

Rau, E.E. and G.A. Langer: Dissociation of energetic state and potassium loss from anoxic myocardium.
Am. J. Physiol. 235: H537-H543, 1978.

Redding, V.J. and J.R. Rees: Early changes in collateral flow following coronary artery ligation: the role of the sympathetic nervous system.
Cardiovasc. Res. 3: 219-225, 1968.

Regan, T.J., H.A. Oldewurtel, and P.O. Ettinger: Regional coronary blood flow during ischemia or epinephrine: relation to myocar-

dial potassium and triglyceride.
In: Myocardial blood flow in man. Methods and significance in coronary disease (ed.: A. Maseri). Minerva Medica, Torino, Italy (1972), p. 424-434.

Reimer, K.A., M.M. Rasmussen, and R.B. Jennings: Reduction by propranolol of myocardial necrosis following temporary coronary artery occlusion in dogs.
Circ. Res. 33: 353-363, 1973.

Reimer, K.A., R.B. Jennings, and M.L. Hill: Total ischemia in dog hearts in vitro. 2. High-energy phosphate depletion and associated effects in energy metabolism, cell volume regulation, and sarcolemmal integrity.
Circ. Res. 49: 901-911, 1981.

Reneman, R.S., A.H.M. Jageneau, W. van Gerven, J. Dony, and P. Beirnaert: The radioactive microsphere method for the assessment of regional myocardial blood flow after coronary artery occlusion.
Pfluegers Arch. 353: 337-347, 1975.

Reneman, R.S. and A. Verheyen: The radioactive microsphere method.
Bibl. Anat. 15: 15-19, 1977.

Reneman R.S., A. Verheyen, W. van Gerven, L. Stijnen and A.H.M. Jageneau: The importance of size and diameter distribution of the microspheres for accurate determination of regional myocardial blood flow (MBF).
Bibl. Anat. 15: 30-34, 1977.

Reneman, R.S., A. Verheyen, W. van Gerven, L. Stijnen, and A.H.M. Jageneau: The importance of size and diameter distribution of the microspheres for accurate determination of regional myocardial blood flow (MBF).
Bibl. Anat. 15: 30-34, 1979.

Reneman, R.S., D.W. Slaaf, L. Lindbom, G.J. Tangelder, and K.-E. Arfors: Muscle blood flow disturbances produced by simultaneously elevated venous and total muscle tissue pressure.
Microvasc. Res. 20: 307-318, 1980.

Riemersma, R.A., R.L. Logan, M.F. Russell, and M.F. Oliver: Heparin, free fatty acids and myocardial infarction in man.
J. Mol. Cell. Cardiol. 9 (suppl.): 45, 1977.

Riemersma, R.A., R.L. Logan, and M.F. Oliver: Intralipidheparin in metabolic studies and in vitro lipolysis.
J. Mol. Cell. Cardiol. 9 (suppl.): 45-46, 1977.

Riemersma, R.A. and M.F. Oliver: Raised plasma free fatty acids (FFA). ischemic myocardial metabolism and arrhythmias.
J. Mol. Cell. Cardiol. 11 (suppl. 2): 48, 1979.

Rivas, F., F.R. Cobb, R.J. Bache, and J.C. Greenfield: Relationship between blood flow to ischemic regions and extent of myocardial infarction. Serial measurements of blood flow to ischemic regions in dogs.
Circ. Res. 38: 439-447, 1976.

Rognoni, F. and V. Vigano: Experimental research on metabolic effects of heparin and

myocardial activity.
Artery 3: 180-187, 1977.

Ross, M.A. and D.D. Streeter Jr.: Nonuniform subendocardial fiber orientation in the normal macaque left ventricle.
Eur. J. Cardiol. 3: 229-247, 1975.

Rothlin, M.E. and R.J. Bing: Extraction and release of individual free fatty acids by the heart and fat depots.
J. Clin. Invest. 40: 1380-1386, 1961.

Rouleau, J., L.E. Boerboom, A. Surjadhana, and J.I.E. Hoffman: The role of autoregulation and tissue diastolic pressures in the transmural distribution of left ventricular blood flow in anesthetized dogs.
Circ. Res. 45: 804-815, 1979.

Rovetto, M.J.: Energy metabolism in the ischemic heart.
Tex. Rep. Biol. Med. 39: 397-409, 1979.

Rudolph, A.M., and M.A. Heymann: The circulation of the fetus in utero. Methods for studying distribution of blood flow, cardiac output and organ blood flow.
Circ. Res. 21: 163-184, 1967.

Rushmer, R.F., D.L. Franklin, and R.M. Ellis: Left ventricular dimensions recorded by sonocardiometry.
Circ. Res. 34: 1-8, 1956.

Rubio, R., and R.M. Berne: Release of adenosine by the normal myocardium in dogs and its relationship to the regulation of coronary resistance.
Circ. Res. 25: 407-415, 1969.

Russo, J.V., S. Margolis, C.C. Friesinger, and R.S. Ross: Heparin and ventricular arrhythmias after myocardial infarction.
The Lancet ii: 1271-1275, 1970.

Rutenberg, H., J.C. Pamintuan, and L.A. Soloff: Serum free fatty acids and their relation to complications after acute myocardial infarction.
The Lancet ii: 559-564, 1969.

Schamhardt, H.C.: Regional myocardial perfusion and performance.
Ph.D.-thesis, Erasmus University, Rotterdam (1980).

Schamhardt, H.C., P.D. Verdouw, T.M. van der Hoek, and P.R. Saxena: Regional myocardial perfusion and wall thickness and arteriovenous shunting after ergotamine administration to pigs with a fixed coronary stenosis.
J. Cardiovasc. Pharmacol. 1: 673-687, 1979.

Schaper: W., The physiology of the collateral circulation in the normal and hypoxic myocardium.
In: Rev. Physiol. Biochem. Exp. Pharmacol. (eds.: E. Helmreich et al). Springer Verlag, Berlin-Heidelberg-New York, pp. 102-145 1971a (chapter 63).

Schaper W., (ed.): The collateral circulation of the heart. North-Holland Publ. Co., Amsterdam-London-New York (1971b).

Schaper, W., P. Lewi, and A.H.M. Jageneau:

The determinants of the rate of change of the left ventricular pressure (dP/dt).
Arch. Kreislaufforsch. 46: 27-41, 1965.

Schaper, W., J. Mulch, B. Winkler, and W. Schaper: Ultrastructural, functional and biochemical criteria for estimation of reversibility of ischemic injury: a study on the effects of global ischemia on the isolated dog heart.
J. Mol. Cell. Cardiol. 11: 521-543, 1979.

Scheuer, J. and S.W. Stezoski: Protective role of increased myocardial glycogen stores in cardiac anoxia in the rat.
Circ. Res. 27: 835-849, 1970.

Schosser, R., K.-E. Arfors, and K. Messmer: MIC-II: a program for the determination of cardiac output, arterio-venous shunt and regional blood flow using the radioactive microsphere method.
Comput. Progr. Biomed. 9: 19-39, 1979.

Segre: G., Radial particle displacements in Poiseuille flow of suspensions.
Nature 189: 209-210, 1961.

Shug, A.L., J.H. Thomsen, J.D. Folts, N. Bittar, M.I. Klein, J.R. Koke, P.J. Huth: Changes in tissue levels of carnitine and other metabolites during myocardial ischemia and anoxia.
Arch. Biochem. Biophys. 187: 25-33, 1978.

Snoeckx, L.H., J.L. Verheyen, A. van de Water, P. Lewi, and R.S. Reneman: On-line computation of cardiac output with the thermodilution method using a digital minicomputer.
Cardiovasc. Res. 10: 556-564, 1976.

Sobel, B.E., P.B. Corr, A.K. Robinson, R.A. Goldstein, F.X. Witkowski, and M.S. Klein: Accumulation of lysophosphoglycerides with arrhythmogenic properties in ischemic myocardium.
J. Clin. Invest. 62: 546-553, 1978.

Spaan, J.A.E., N.P.W. Breuls and J.D. Laird: Diastolic systolic coronary flow differences are caused by intramyocardial pump action in the anesthetized dog.
Circ. Res. 49: 584-593, 1981.

Spiro, D. and E.H. Sonnenblick: The structural basis of the contractile process in heart muscle under physiological and pathological conditions.
Progr. Cardiovasc. Dis. 7: 295, 1965.

Spitzer, J.J.: Effect of lactate infusion on canine myocardial free fatty acid metabolism in vivo.
Am. J. Physiol. 226: 213-217, 1974.

Stam, H. and W.C. Huelsmann: Release of lipolytic products from rat heart. Hormonal stimulation, intracardiac origin and pharmacological modification.
Biochem. Inter. 2: 477-484, 1981.

Stegal, J.F., M.B. Kardon, H.L. Stone, and V.S. Bishop: A portable, simple sonomicrometer.
J. Appl. Physiol. 23: 289-293, 1967.

Stetten, M.R.: Metabolism of inorganic pyrophosphate. I. Microsomal inorganic pyrophosphate phosphotransferase of rat liver.
J. Biol. Chem. 239: 3576-3583, 1964.

Streeter Jr, D.D., H.M. Spotnitz, D.P. Patel, J. Ross Jr., and E.H. Sonnenblick: Fiber orientation in the canine left ventricle during diastole and systole.
Circ. Res. 24: 339-347, 1969.

Streeter Jr., D.D., R.N. Vaishanov, D.J. Patel, H.R. Spotnitz, J. Ross Jr., and E.H. Sonnenblick: Stress distribution in the canine left ventricle during diastole and systole.
Biophys. J. 10: 345-363, 1970.

Streeter Jr., D.D. and W.T. Hanna: Engineering mechanics for successive states in canine left ventricular myocardium . II Fiber angle and sarcomere length.
Circ. Res. 33: 656-664, 1973.

Takano, S.: Genetic studies on the arrhythmia in acute myocardial infarction with special reference to serum free fatty acid level.
Jpn. Circ. J. 40: 287-297, 1976.

Takenaka, F. and M. Higuchi: High-energy phosphate contents of subepicardum and subendocardium in the rat treated with isoproterenol and some other drugs.
J. Mol. Cell. Cardiol. 6: 123-135, 1974.

Tatooles, C.J. and W.C. Randall: Local ventricular bulging after acute coronary occlusion.
Am. J. Physiol. 201: 451-456, 1961.

Tennant, R. and C.J. Wiggers: The effect of coronary occlusion on myocardial contraction.
Am. J. Physiol. 112: 351-361, 1935.

Ten Velden, G.H.M.: Heat production of the canine left ventricle.
Ph.D.-thesis, Free University, Amsterdam (1982).

Ten Velden, G.H.M., G. Elzinga, and N. Westerhof: Left ventricular energetics: heat loss and temperature distribution of canine myocardium.
Circ. Res. 50: 63-73, 1982.

Theroux, P., D. Franklin, J. Ross Jr., and W.S. Kemper: Regional myocardial function during acute coronary artery occlusion and its modification by pharmacologic agents in the dog.
Circ. Res. 35: 896-908, 1974.

Theroux, P., J. Ross Jr., D. Franklin, W.S. Kemper, and Sh. Sasayama: Regional myocardial function in the conscious dog during acute coronary occlusion and responses to morphine, propranolol, nitroglycerin, and lidocaine.
Circulation 53: 302-314, 1976.

Theroux, P., J. Ross Jr., D. Franklin, J.W. Covell, C.M. Bloor, and S. Sasayama: Regional myocardial function and dimensions early and late after myocardial infarction in the unanesthetized dog.
Circ. Res. 40: 158-165, 1977.

Torrent-Guasp, F.: The cardiac muscle. Juan

March Foundation, Madrid (1973).

Tripp, M.R., S. Einzig, J.J. Leonard, A. Gerasch, C.R. Swayze, M.D. Manuel and I.J. Fox: Recent developments in the measurement of cardiac output and regional blood flow using indocyanine green and microspheres. Med. Instrum. 11: 160-165, 1977.

Trump, B., W. Mergner, M. Won Kahng, and A. Saladino: Studies on the subcellular pathophysiology of ischemia. Circulation 1: 17-26, 1976.

Tyberg, J.V., J.S. Forrester, H.L. Wyatt, S.J. Goldner, W.W. Parmley, and H.J.C. Swan: An analysis of segmental ischemic dysfunction utilizing the pressure-length loop. Circulation 49: 748-754, 1974.

Utley, J., E.L. Carlson, J.I.E. Hoffman, H.M. Martinez, and G.D. Buckberg: Total and regional myocardial blood flow measurements with 25 m, 15 m, 9 m and filtered 1-10 m diameter microspheres and antipyrine in dogs and sheep. Circ. Res. 34: 391-405, 1974.

Vary, T.C., D.K. Reibel, and J.R. Neely: Control of energy metabolism of heart muscle. Ann. Rev. Physiol. 43: 419-430, 1981.

Vary, T.C., E.T. Angelakos, and S.W. Schaffer: Relationship between adenine nucleotide metabolism and irreversible ischemic tissue damage in isolated perfused rat heart. Circ. Res. 45: 218- 225, 1979.

Vasdev, S.C., G.P. Biro, R. Narbaitz, K.J. Kako: Membrane changes induced by early myocardial ischemia in the dog. Can. J.Biochem. 58: 1112-1119, 1980.

Verdouw, P.D., H.C. Schamhardt, W.J. Remme, and J.W. de Jong: Antiarrhythmic, metabolic and hemodynamic effects of org 6001 (3alfa-amino5alfa -androstan-2beta-OL-17-one-hydrochloride) after coronary flow reduction in pigs. J. Pharmacol. Exp. Ther. 204: 634-644, 1978.

Verdouw, P.D., W.J. Remme, J.W. de Jong, and W.A.P. Breeman: Myocardial substrate utilization and hemodynamics following repeated coronary flow reduction in pigs. Basic Res. Cardiol. 74: 477-493, 1979.

Vik-Mo, H., R.A. Riemersma, O.D. Mjøs, and M.F. Oliver: Effect of myocardial ischemia and antilipolytic agents on lipolysis and fatty acid metabolism in the in situ dog heart. Scand. J. Clin. Lab. Invest 39: 559-569, 1979.

Vik-Mo, H. and O.D. Mjøs: Influence of free fatty acids on myocardial oxygen consumption and ischemic injury. Am. J. Cardiol. 48: 361-365, 1981.

Visser, M.P., M.T.A. Krill, A.M.M. Muytjens, G.M. Willems, and W.Th. Hermens: Distribution of enzymes in dog heart and liver; significance for assessment of tissue damage from data on plasma enzyme activities. Clin. Chem. 27, 1845-1850, 1981.

Vliers, A.C.A.P., K.R. Visser, and W.G. Zijlstra: Analysis of indicator distribution in the determination of cardiac output by thermal dilution. Cardiovasc. Res. 7: 125-132, 1973.

Vusse, G.J. van der: Are free fatty acids harmful for the heart¤ J. Drug Res. 000-000, 1982.

Vusse, G.J., van der, H. van Belle, W. van Gerven, R. Kruger, and R.S. Reneman: Acute effect of fentanyl on hemodynamics and myocardial carbohydrate utilization and phosphate release during ischemia. Br. J. Anesth. 51: 927-935, 1979.

Vusse, G.J. van der, F.W. Prinzen, W.A. Coumans, R. Kruger, C. Verlaan, and R.S. Reneman: Assessment of myocardial ischemia using hemodynamic and biochemical variables with special reference to elevated arterial free fatty acid concentration by heparin. Adv. Clin. Cardiol. 1: 407-420, 1980a.

Vusse, G.J. van der, Th.H.M. Roemen, and R.S. Reneman: Assessment of fatty acids in dog left ventricular myocardium. Biochim. Biophys. Acta 617: 347-352, 1980b.

Vusse, G.J. van der, F.W. Prinzen, and R.S. Reneman: Myocardial substrate uptake. Bibl. Anat. 20: 497-502, 1981a.

Vusse, G.J. van der, Th.H.M. Roemen, F.W. Prinzen, and R.S. Reneman: The concentration of non-esterified fatty acids in biopsies from normoxic dog myocardium. Basic Res. Cardiol. 76: 389-393, 1981b.

Vusse, G.J. van der, and R.S. Reneman: Glycogen and lipids (endogenous substrates). in: Cardiac metabolism. (eds. M.I.M. Noble and A.J. Drake. published by John Wiley and Sons Ltd, Chichester, England, 1982)

Vusse, G.J. van der, Th.H.M. Roemen, F.W. Prinzen, W.A. Coumans, and R.S. Reneman: Uptake and tissue content of fatty acids in dog myocardium under normoxic and ischemic conditions. Circ. Res. 50: 000-000, 1982.

Weber, K.T. and J.S. Janicki: Myocardial oxygen consumption: the role of wall force and shortening. Am. J. Physiol. 233: H421-H430, 1977.

Weglicki, W.B., I. Owens, C.W. Urschel, J.R. Serur, and E.H. Sonnenblick: Hydrolysis of myocardial lipids during acidosis and ischemia. Recent. Adv. Stud. Cardiac Struct. Metab. 3: 781-793, 1973.

Weintraub, W.S., S. Hattori, J.B. Agarwal, M.M. Bodenheimer, V.S. Banka, and R.H. Helfant: The relationship between myocardial blood flow and contraction by myocardial layer in the canine left ventricle during ischemia. Circ. Res. 48: 430-438, 1981.

Weishaar, R., J.S.M. Sarma, Y. Maruyama, R. Fischer, and R.J. Bing: Regional blood flow, contractility and metabolism in early myocardial infarction.

Cardiology 62: 2-20, 1977.

Weishaar, G.V. K. Tschurtschenthaler, K. Ashikawa, and R.J. Bing: The relationship of regional coronary blood flow to mitochondrial function during reperfusion of the ischemic myocardium.
Cardiology 64: 350-365, 1979.

Weiss, H.R.: Effect of coronary artery occlusion on regional arterial and venous $O_2$ saturation, $O_2$ extraction, blood flow and $O_2^2$ consumption in the dog heart.
Circ. Res. 47: 400-408, 1980.

Weiss, H.R., J.A. Neubauer, J.A. Lipp, and A.K. Sinha: Quantitative determination of regional oxygen consumption in the dog heart.
Circ. Res. 42: 394-402, 1978.

Whitmer, J.T., J.A. Idell-Wenger, M.J. Rovetto, and J.R. Neely: Control of fatty acid metabolism in ischemic and hypoxic hearts.
J. Biol. Chem. 253: 4305-4309, 1978.

Williamson, J.R.: Glycolytic control mechanisms. II. Kinetics of intermediate changes during the aerobic- anoxic transition in perfused rat heart.
J. Biol. Chem. 241: 5026-5036, 1966.

Williamson, J.R.: Kinetic studies of epinephrine effects in the perfused heart.
Pharmacol. Rev. 18: 205-210, 1966.

Williamson, J.R., S.W. Schaffer, A. Ford, and B. Safer: The cellular basis of ischemia and infarction, contribution of tissue acidosis to ischemic injury in the perfused rat heart.
Circulation 53, Suppl. I: I-3-I-14, 1976.

Wilson, M.F., W.M. Caldwell, J.M. Gibson, and S.J. Cho: Gauging systems to measure internal organ dimensions.
Am. J. Med. Elctron. 2: 328-334, 1963

Winbury, M.M., B.B. Howe: Stenosis: regional ischemia and reserve.
In: Perspectives in cardiovascular research vol. 3. Ischemic myocardium and antianginal drugs. Eds. M.M. Winbury and Y Abiko, Raven Press, New york, N.Y., 1979, pp. 55-76.

Wollenberger, A. and L. Shahab: Anoxia-induced release of noradrenaline from the isolated perfused heart.
Nature 207: 88-89, 1965.

Wollenberger, A. and E.G. Krause: Metabolic control characteristics of acutely ischemic myocardium.
Am. J. Cardiol. 22: 349-359, 1968.

Wuesten, B., D.D. Buss, H. Deist, and W. Schaper: Dilatory capacity of the coronary circulation and its correlation to the arterial vasculature in the canine left ventricle.
Basic Res. Cardiol. 72, 636-650, 1977.

Wyatt, H.L., J.S. Forrester, J.V. Tyberg, S. Goldner, S.E. Logan, W.W. Parmley, and H.J.C. Swan: Effect of graded reductions in regional coronary perfusion on regional and total cardiac function.
Am. J. Cardiol. 36: 185-192, 1975.

Yagi, K. and R. Mase: Possible compartmentation of adenine nucleotides in a coupled reaction system composed of F-actomyosinadenosinetriphosphatase and creatine kinase.
In: Molecular biology of muscular biology of muscular contraction (eds.: S. Ebashi, F. Osawa, T. Sekine and Y. Tonomura). Elsevier Publ. Co., Amsterdam, 1965, pp. 109-123.

Yamazaki, N., Y.Suzuki, T. Kamikawa, K. Ogawa, K. Mzutani, K. Kakizawa, and M. Yamamoto: Arrhythmogenic effects of acute free fatty acid mobilization on ischemic heart.
Recent Adv. Stud. Cardiac Struct. Metab 12: 271-277, 1978.

Yipintsoi, T., W.A. Dobbs, P.D. Scanlon, T.J. Knopp, and J.B. Bassingthwaighte: Regional distribution of diffusible tracers and carbonized microspheres in the left ventricle of isolated dog hearts.
Circ. Res. 33: 573-587, 1973.

Yoran, C., J.W. Covell and J. Ross: Structural basis for the ascending limb of left ventricular function.
Circ. Res. 32: 297-303, 1973.

Zucker, I.H. and J.P. Gilmore: Atrial receptor discharge during acute coronary occlusion in the dog.
Am. J. Physiol. 227: 360-363, 1974.

NAWOORD

De studie, die aan dit proefschrift ten grondslag ligt, is
begonnen als een projekt binnen de werkgemeenschap hartfunktie
van de Stichting voor Medisch Wetenschappelijk Onderzoek
(FUNGO), met als projektleider prof. dr. R.S. Reneman. Het
onderzoek is uitgevoerd aan de Rijksuniversiteit Limburg,
binnen het kader van het deelprojekt Myocard onder leiding van
prof. dr. R.S. Reneman.
Een proefschrift staat op naam van een persoon, maar bijdragen
zijn geleverd door velen. Al diegenen, die op enige wijze een
bijdrage geleverd hebben aan de tot stand koming van dit werk-
stuk wil ik hierbij hartelijk danken. Zonder daarbij iemand te
kort te willen doen, wil ik een aantal met name noemen.
Het uitvoeren van dit onderzoek, waarbij verschillende facetten
bestudeerd zijn van de drie-eenheid doorbloeding-metabolisme-
mechanica van de hartspier, was alleen mogelijk dankzij grondi-
ge voorbereiding door, en goede samenwerking met Rob Reneman,
Ger van der Vusse en Theo Arts.
Mijn dank gaat tevens uit naar Jasper Scholte, Mark Noble, Gijs
Elzinga en Foppe ten Hoor, die met hun kritische kommentaren
een waardevolle bijdrage geleverd hebben aan de totstandkoming
van dit proefschrift.
Wil Coumans, Theo Roemen, Mat Rousch en Peter Willemsen hebben
met grote nauwkeurigheid de chemische bepalingen verricht, en
hebben geassisteerd bij de experimenten.
Het slagen van deze experimenten is voor een belangrijk deel te
danken aan de biotechnische bijstand van Inger Simons-Achter-
berg, Ruud Kruger, Cees Verlaan en Theo van de Nagel.
Els Geurts heeft het leeuwendeel van het typwerk verricht. Ik
ben haar dankbaar voor de grote snelheid waarmee zij steeds
weer wijzigen aanbracht in tekst, en regel- en tabelindelingen.
Even dankbaar ben ik voor de hulp van Bea Leliveld en Erik van
der Veen bij het toch nog tijdig voltooien van dit proef-
schrift.

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