

**THE LOCAL CONTROL  
OF  
CORONARY BLOOD FLOW**

by

**Xiao Fang XU**

M.B. (Shanghai Medical University)

Department of Medicine

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## DECLARATION

I declare that the investigations described in this thesis constitute my own work. This thesis contains no material which has been accepted for the award of any other higher degree or graduate diploma in any tertiary institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text.

Signed: 徐能芳

Date: May, 1991

## ABSTRACT

The mechanism of local metabolic regulation of coronary blood flow is still unclear. It is still unknown which control system – an "open-loop feedback control" or a "close-loop control" – operates in the coronary circulation and whether common mechanisms exist in this control. To separate the putative chemicals proposed to be physiological regulators in the coronary bed, the time courses of coronary resistance change in response to either increased cardiac work or hypoxia have been examined, before and after  $\alpha$ ,  $\beta$  and adenosine inhibition.

An acute open-chest sheep model was used for this investigation. The sheep were anaesthetised with sodium pentobarbitone and instrumented for circumflex coronary artery and pulmonary artery flows, left ventricular, proximal aortic and coronary sinus pressure. Continuous coronary sinus oxygen saturation was measured by a fibre optic technique. Blood samples from coronary sinus and proximal aorta were analysed for blood gases,  $\text{Na}^+$  and  $\text{K}^+$ . An occluding band was placed around the proximal aorta to produce variable increments of aortic pressure for variable periods. Low oxygen gases (5% $\text{O}_2$ , 5% $\text{CO}_2$  and 90% $\text{N}_2$ ) were used to produce systemic hypoxia. Phentolamine, propranolol and 8-phenyltheophylline were used as  $\alpha$ ,  $\beta$  and adenosine blockers respectively, and nonadrenaline and isoprenaline (injected into left ventricle) and intra-coronary injection of adenosine were used to test the efficacy of the blockers. Continuous coronary resistance was calculated throughout each intervention.

The recovery of coronary resistance to normal after the change of the afterload of the left ventricle had a constant half life of  $4.7 \pm 0.2$  seconds. The half life was not related to either the degree or the duration of increased work.  $\alpha$ ,  $\beta$  and adenosine inhibitors did not alter the half life, but produced more profound changes in coronary

resistance due to increased cardiac work. The half life of exogenous adenosine was about 26 seconds.

During the increase of aortic pressure the coronary sinus oxygen saturation varied significantly both above and below the steady state unpredictably, but with systemic hypoxia there was vasodilation during which the oxygen level in the coronary sinus was closely linked to coronary resistance with a small "hysteresis" when the hypoxia was reversed. After adenosine blockade, the coronary resistance and oxygen supply were more closely linked.

The results suggest an open loop control system with a short acting mediator whose half life is the same for varying periods and degrees of increased work, but very much shorter than that of adenosine. The metabolic control of coronary flow is not dependent on the oxygen requirements of the muscle. Multiple factors may be involved in the local coronary vasoregulation. Current data do not support adenosine as the common factor in metabolic vasodilation.



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## Abbreviation Glossary

<b>Ach</b>	Acetylcholine
<b>ADO</b>	Adenosine
<b>aop</b>	Aortic Pressure
<b>cAMP</b>	Cyclic 3',5'-AMP
<b>CBF</b>	Coronary Blood Flow
<b>CS</b>	Coronary Sinus
<b>csp</b>	Coronary Sinus Pressure
<b>cr</b>	Calculated Mean Diastolic Coronary Resistance
<b>cw</b>	Cardiac Work
<b>d.aop</b>	Diastolic Aortic Pressure
<b>DF</b>	Degree of Freedom
<b>EDRF</b>	Endothelium-Derived Relaxing Factor
<b>8-PT</b>	8-phenyltheophylline
<b>IMP</b>	Inosine Monophosphate
<b>pqm</b>	Mean Pulmonary Flow
<b>cqm</b>	Mean Coronary Circumflex Blood Flow
<b>cqd</b>	Mean diastolic Coronary Circumflex Blood Flow
<b>HR</b>	Heart Rate
<b>LAD</b>	Left Anterior Descending Artery
<b>LVDP</b>	Left Ventricular Diastolic Pressure
<b><math>\dot{MVO}_2</math></b>	Myocardial Oxygen Consumption
<b>NO</b>	Nitric Oxide
<b>NOLA</b>	Nitro-L-arginine
<b>P(t)O<sub>2</sub></b>	Tissue Oxygen Tension
<b>PVA</b>	Left Ventricular Pressure-Volume Area
<b>R.H.H.</b>	Royal Hobart Hospital
<b>s.aop</b>	Systolic Aortic Pressure
<b>SEM</b>	Standard Error of Mean
<b>s.LVP</b>	Systolic Left Ventricular Pressure
<b>SD</b>	Standard Deviation
<b>SO<sub>2</sub></b>	Oxygen Saturation
<b>SOD</b>	Superoxide Dismutase
<b><math>\Delta p</math></b>	Percentage Change of Systolic Aortic Pressure



# **CHAPTER 1**

## **INTRODUCTION**

## 1.1 INTRODUCTION

Cardiovascular diseases remain one of the major causes of morbidity and mortality in modern society. The scientific community has paid increasing attention to the role of vasoactivity of blood vessels which may affect or accompany such diseases. To understand the pathophysiological mechanisms that are responsible for the clinical manifestations of various cardiac states, it is necessary to gain a thorough understanding of the physiological mechanisms that operate in the healthy individual.

The circulation system has the principal function of transport, to deliver the oxygen and nutrients necessary for tissue metabolism and to remove the waste products and heat resulting from the metabolic processes. Each organ will place different demands on the circulation to function effectively. In numerous examples the system has shown how effectively the function of the system is altered to meet the demand and to function smoothly. The feedback loops involved in regulatory activities may comprise local mechanisms of humoral or metabolic origin contained within the organ itself, and neural mechanisms mediated at the spinal cord level or by the higher centres in the brain.

It has been observed that during exercise there is a 15- to 20-fold increase in blood flow to skeletal muscles, and a 3- to 4-fold increase to the myocardium, but a decrease in blood flow to the kidney, splanchnic area and nonexercising muscle. With changes in emotional state such as quiet, stress and sleep, there would be a different supply of blood to different organs, mainly dependent on the demand of each organ. For the exercising situation, with a rapid increase in energy requirements, relatively rapid circulatory adjustments are essential in order to meet the increased need for  $O_2$  and nutrients by the exercising muscle. Also important is the need to remove the end products of metabolism such as  $CO_2$  and lactic acid and to dissipate the excess heat

produced by the reactions. How the circulatory system meets these increased demands is still an enigma.

Although the mechanisms of vasoregulation may be similar throughout the body, the tissues served by the vasculature are heterogeneous and provide very specific functions. Different organs will need different systems of both systemic regulation and local regulation. In this thesis, only the local control of blood flow in coronary circulation will be discussed. The term "local control of blood flow" means the blood flow to certain organ can be regulated smoothly and effectively within organ itself to supply the demand of the organ without evoking the autonomic system. Investigators have tried to explain this phenomenon in many ways. However, none of the explanations satisfy all of the observed phenomena. Researchers in this area used one term "autoregulation" to describe these phenomenon. In order to specify the auto-regulation due to different causes, most recent researchers use "autoregulation" only for the flow re-adjustment due to the change of driving pressure of blood flow and use another term "metabolic vasodilation" for the flow re-adjustment due to the change of the metabolic state when there is no alteration of perfusion pressure.

The phenomenon of "flow re-adjustments" of the coronary system shows that the coronary circulation can always supply enough blood to meet the demands of the heart within a certain range without requiring the central nervous control. According to a general feedback control model, there are two control systems. As the heart metabolic status changes, there are two ways for the coronary circulation to be adjusted in order to meet the demands of the heart under the new metabolic situation. One is that the muscle may adjust the release of some substances, with the property of coronary vasodilation into the interstitium of the tissue to increase the coronary blood flow (CBF). This system can be called "open-loop feedback control". In this case very often the system will overshoot, which means that coronary circulation may supply more blood flow than it actually needs for any particular situation. The

alternative is that a change of heart work induces a different metabolic status which is detected by the system which then releases some vasoactive substances into the interstitium to regulate CBF to keep the metabolic status constant. This can be called "close-loop feedback controlling system". Which system actually controls coronary circulation is unclear, but most researchers favour the close-loop feedback controlling system.

In addition to not knowing which controlling system operates in the coronary circulation, we also do not know the exact metabolite or even whether a single metabolite or several metabolites are involved. Reports in the literature are conflicting. Several compounds such as adenosine, some of the prostaglandins and endothelial dependent relaxing factor (EDRF) have a temporal relationship to vasodilation.

This thesis sets out to further examine whether common mechanisms exist in local control of CBF and to collect further data to separate the putative chemicals proposed to be physiological regulators in coronary vasoregulation. To do so, I will emphasize vasoregulation related to increased cardiac effort and decreased oxygen supply.

The **hypotheses** for this work are:

- *There are common mechanisms in the local regulation of CBF after the changes of cardiac work and hypoxia.*
- *The control of CBF due to the changes of metabolic states of the heart is independent of the autonomic nervous system.*
- *Oxygen is an end control factor in the regulation of CBF.*
- *Adenosine is not the end control factor in the regulation of CBF.*

The general **aims** are:

- *to determine the time course of changes in coronary resistance in response to increased cardiac work, both before and after  $\alpha$ ,  $\beta$  and adenosine inhibition;*
- *to elucidate whether common mechanisms exist in the local regulation of coronary circulation and to examine which control system, "an open-loop feedback control" or "a close-loop control" operates in coronary circulation and*
- *to assess the relationship of work done and severity of hypoxia and metabolic state.*

## 1.2 ORGANISATION OF THESIS

This thesis is divided into 9 chapters, a bibliography and four appendices. Chapter 1 contains this introduction. In chapter 2, I examine how the various theories were postulated historically and how they were tested in different regional circulations throughout the body. Chapter 2 also outlines the development of studies of mechanisms in the local control of circulation. Chapter 3 systematically reviews the roles of oxygen and adenosine in the local control of coronary circulation from published work. In chapter 4, the methods and materials used for this work are discussed. In chapters 5, 6, 7 and 8, I present, discuss and analyze each study separately and compare my results with those of others. Chapter 9 synthesizes my findings, extends understanding in this field, gives conclusions, and postulates future work.

## **CHAPTER 2**

### **HISTORICAL REVIEW**

## 2.1 INTRODUCTION

Autoregulation of blood flow, in the broad sense, can be defined as the capability of an organ to regulate its blood supply in accordance with its metabolic need. But the usual, more exact definition of autoregulation is the intrinsic ability of the organ to maintain its blood flow relatively constant despite the changes in perfusion pressure [Johnson, 1964; Feigl, 1983; and Dole, 1987]. Metabolic regulation refers to the vasoregulation due to the changes of the metabolic need within the organ itself.

The earliest observation of this phenomenon was by Jone in 1852, who discovered that rhythmical vasoconstrictions in the bat wing veins are dependent on the internal pressure. He was also the first to describe myogenic activity of blood vessels. In 1902, Bayliss observed that as the arterial pressure rises, the hindlimb arteries are distended passively and when the blood pressure falls again, they constrict much below their previous level and only gradually return to that state. He also found that as the arterial pressure diminishes, the limb arteries relax. Those reactions are independent of the central nervous system and can be obtained both in normal condition and excited conditions in arteries. Thus he postulated that the blood flow regulation described by Jone as myogenic in nature is present in arterial vessels as well [Bayliss, 1902]. To this day, some researchers still call this the Bayliss phenomenon.

After the first description of autoregulation on dog's kidneys by Rein [1931], research in this area was quiet for nearly twenty years, with only a few reports appearing to support Bayliss' hypothesis. Forbes and co-workers [1937] showed the pial arterioles constricted in response to an elevation of arterial pressure and dilated following a reduction. Selkurt [1946] noted that, at an arterial pressure above 80mmHg, local arterial pressure changes had little effect on flow. Folkow [1949] reported that an elevation of the arterial perfusion pressure caused an initial increase in

flow followed by a return toward control levels. Although later workers were able to explain this result by either the myogenic or the metabolic theories, Folkow favoured the myogenic theory. Folkow's study was important because:

- *he demonstrated that denervated organs possess vascular tone;*
- *his studies examined the origin of the vascular tone in a quantitative manner; and*
- *the experiments were designed to overcome the objections that had been raised by earlier critics of Bayliss [Johnson, 1977].*

As the techniques used for circulation research developed in the 1950's, autoregulation and metabolic regulation became the object of serious research as part of an intensified interest in the local mechanism of blood flow regulation. Investigators proposed many explanations for their experimental results with much controversy and few uncontested conclusions. In 1963, a symposium on this topic provided a detailed view of the field at that time, and recognized four important mechanisms of autoregulation. They are myogenic, metabolic, tissue pressure and tubulo-glomerular feedback [Johnson, 1964].

Briefly, the myogenic theory is that the stretch due to an increased arterial pressure causes the arteriolar smooth muscle to constrict in order to keep blood flow relatively constant at the microvascular level. Most research on the myogenic response has been performed on isolated vessels from skeletal muscle. In this area, many experiments have been done using rabbit ear arteries by Speden and his colleagues in the Department of Physiology of the University of Tasmania. They observed that isolated segments of active ear arteries from rabbits reacted to distension by pressure jumps from 60mmHg to 100mmHg with a counteracting constriction which took 1-2mins to achieve near-completion and was not dependent upon a functional sympathetic innervation [Speden, 1984; 1986]. In later experiments, they showed that active ear arteries possessed myogenic mechanisms which had the capacity to



minimize distension of active ear arteries against pulsatile pressures of physiological frequency, mean pressure and pulse amplitude [Speden, 1987]. They also measured the ear artery myogenic response to rapid and gradually induced pressure jumps in either the presence or the absence of the endothelium. The results were not significantly different, leading to the conclusion that the myogenic response is independent of the endothelium in active isolated pressurized rabbit ear arteries [McLeod, 1989]. A few studies have used cerebral and renal vessels. One study has shown that diastolic pressure increase resulted in a brief and small coronary vasoconstriction response in the awake dogs [Sadick, 1987]. After excluding the involvement of a neurogenic mechanism and an increase in CBF which might increase the washout of vasodilating metabolites<sup>1</sup>, the authors attributed the response to the myogenic mechanism.

The metabolic hypothesis proposes that flow is relatively constant because in vascular smooth muscle either constriction or dilation is controlled by metabolic substances which are regulated by organ demand. In this area research has been done in many organs including brain, heart, kidney, and skeletal muscle. Through those experiments, a number of putative metabolic substances have been conjectured: oxygen, carbon dioxide, hydrogen, potassium, adenosine, adenine nucleotides, magnesium, osmotic, prostaglandins, free radical and endothelial substances [Johnson 1964; 1986; Berne 1979; Feigl 1983; Dole 1987]. Many investigators have set up a series of studies to examine each candidate carefully. Unfortunately none of the proposed metabolites can explain all the phenomena. It has been difficult to separate myogenic and metabolic mechanisms of autoregulation in the heart because changes in perfusion pressure (myogenic stimulus) very often result in increased work which is also associated with changes in blood flow (metabolic stimulus).

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<sup>1</sup>This increase of the washout of vasodilating metabolites may be responsible for the vasoconstriction following the increase of perfusion pressure (metabolic instead of myogenic theory).

The tissue pressure hypothesis is based on the observation that an increase in perfusion pressure induced an increase in the permeability of vessel walls, thus increasing ultrafiltration, resulting in an increase in tissue pressure. This hypothesis may be important for those vessels in organs which have a stiff capsule such as renal arterioles, because the increase of tissue pressure, that is interstitial pressure, would tend to collapse small veins and some arterioles if the organs were encapsulated [Johnson, 1986].

The tubulo-glomerular feedback theory suggests that an increase in arterial pressure leads first to increased glomerular filtration, secondly to increased solute and concentration of electrolytes such as  $\text{Na}^+$  in the tubular fluid. It is suggested that this acts on the macula densa which are considered to sense changes in the tubular fluid and may release a constrictor, which restores glomerular filtration to close to the original level by decreasing flow [Johnson, 1986].

## **2.2 LOCAL VASOREGULATION IN REGIONAL CIRCULATION**

### **2.2.1 Cerebral Circulation**

Cerebral autoregulation refers to a cerebral blood flow that remains relatively constant in response to the change of cerebral perfusion pressure. Cerebral blood flow is maintained relatively constant down to an arterial pressure of 60-70mmHg [MacKenzie, 1979; Hernandez, 1978; Fitch, 1975]. As blood pressure was lowered further, below the so-called lower limit of autoregulation, cerebral blood flow decreased despite continued vasodilation of the cerebral resistance vessels [Heistad, 1983]. When the arterial blood pressure is at 40mmHg, maximum vasodilation is reached [Kontos, 1978a; MacKenzie, 1979], suggesting that 40mmHg more accurately represents the lower limit of autoregulation in cerebral circulation. In the brain, because of its heavy dependence on aerobic metabolism and its high resting oxygen consumption [Eklof, 1973], many investigators have proposed the hypothesis

that oxygen levels in tissue are directly and inversely active on the arterial supply. *ie* a low tissue  $pO_2$  causes direct vasodilation. Kontos and co-workers [1978b] demonstrated arterial hypoxia produced marked cerebral arterial vasodilation, which was partially reversed by perfusing the space under the cranial window with artificial cerebrospinal fluid containing 94% oxygen. He suggests two possible mechanisms for this oxygen hypothesis. First, it may be a kind of local reflex. The cerebral vessels have nerves attached to them originating in the brain itself [Falck, 1965; Swanson, 1975]. However it remained controversial because Edvinson and his colleagues [1975] failed to confirm the existence of these nerves. Secondly, cell hypoxia induces the release of vasodilator metabolites which are responsible for vascular smooth muscle relaxation of the pial arterioles by diffusion. Rubio's experiment [1975] failed to support oxygen as a mediator of cerebral autoregulation. However, this conclusion was based on the assumption<sup>1</sup> that adenosine is responsible for the cerebral autoregulation. The results: a) hypoxia caused by ventilating low oxygen gas induced the brain adenosine levels to increase significantly; b) increase in  $pO_2$  from 21 to 29.7mmHg did not produce changes in adenosine levels, leading them to conclude that oxygen is not a direct mediator in cerebral vasoregulation due to the alteration of perfusion pressure.

Winn [1980a; 1980b; 1981] suggested adenosine is a mediator of cerebral vasoregulation. To assess the role of adenosine in the autoregulation of cerebral blood flow, Van Wylen *et al* [1988] measured dialysate adenosine and cerebral blood flow over a range of arterial pressures from 80mmHg to 30mmHg, by using the brain dialysis technique, the hydrogen-clearance technique and an adenosine antagonist. Their results showed that autoregulation existed in all circumstances. Adenosine did not increase until hemorrhaging caused arterial pressure to drop to 50mmHg. With a

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<sup>1</sup>Although an impressive body of experimental data supports this theory, the result is still not conclusive. Thus, I still refer to it as an assumption.

similar reduction in cerebral blood flow, the dialysate adenosine concentration was greater on the side of locally infused theophylline. Thus, they suggest that adenosine is not responsible for cerebral autoregulation at blood pressure > 50mmHg, but may contribute to the decrease in cerebral vascular resistance observed at arterial pressures below the autoregulation range. Wahl *et al* [1979] were unable to detect changes in perivascular H<sup>+</sup> or K<sup>+</sup> over the pressure range of 60-200mmHg despite observing changes in vessel diameter. They pointed out that this can not support the hypothesis that hydrogen or potassium is a mediator of autoregulation under these circumstances. Other researchers found marked vasodilation with very severe increases in arterial perfusing pressure [Kontos, 1978a; MacKenzie, 1976] which seems to contradict the autoregulation hypothesis.

It is well accepted that metabolic and myogenic mechanisms are involved in vasoregulation of cerebral blood flow [Johnson, 1986; Heistad, 1983]. Distinction between these two factors has become very difficult since they often act together. Wagner *et al* [1983] suggested that the dominant mechanism of cerebral autoregulation is metabolic, not myogenic, based on the finding that regional and global cerebral vascular resistance decreased in response to the elevation of cerebral venous pressure. Wei and his colleagues [1984] confirmed Wagner's finding. They observed that myogenic vasoconstriction is not abolished during increases of venous pressure when the metabolic mechanism (dominant mechanism) is eliminated by increased local supply of oxygen. It is still possible that cerebral autoregulation is mediated solely by a myogenic mechanism and that metabolic factors come into play only when the oxygen supply-to-demand ratio is greatly reduced. The predominance of one over the other depends on the initiating stimulus [Van Wylen, 1988]. To date, the precise mechanism of local cerebral vasoregulation is not clear.

### 2.2.2 Skeletal Circulation

In skeletal muscle, two mechanisms responsible for local vasoregulation have been proposed: the myogenic hypothesis [Folkow, 1964; Johnson, 1986] and the metabolic hypothesis [Berne, 1964; Haddy, 1975; 1978]. Arterial pulsatile pressure induces constriction in skeletal muscle vessels [Rovick, 1964]. Transient stretch caused by the arterial pulse pressure induces an especially pronounced response in the distal arteriole and may be an important determinant in the basal vascular tone [Grande, 1977]. In the isolated rabbit ear resistance arteriole, considerable evidence was obtained that stretch-dependent tone may be similar to basal vascular tone which responds to the change in the level of transmural pressure [Hwa, 1986]. Some researchers have presented evidence which indicates that oxygen plays a role in the blood flow control in skeletal muscle [Granger, 1973; Prewitt, 1976]. Granger [1973] developed a mathematical model which showed the correlation between degree of autoregulation and venous oxygen concentration. The major point of discussion between them is the question; by what mechanism does oxygen affect vascular resistance. Two feedback mechanisms have been proposed as follows:

- The vasodilator theory [Imai, 1964; Rubio, 1969; Pittman, 1973; Gorczynski, 1978]

*The change of oxygen state causes the release of some metabolites. Most investigators who are in favour of this theory believe the metabolite is adenosine, from parenchymal cells which moves into the interstitial spaces at a rate which is inversely proportional to the  $O_2$  supply : demand ratio.*

- The direct effect theory [Detar, 1968; Carrier, 1964]

*The intrinsic vasoregulation is mediated through the direct effect of changes in interstitial  $pO_2$  on vascular smooth muscle.*

Both hypotheses have an equally impressive body of experimental results in support. In the cremaster muscle of the rats, Prewitt and co-workers [1976] examined

the effect of  $O_2$  on microcirculatory flow and capillary density over a wide range of bath  $pO_2$ . The change of  $pO_2$  caused inverse changes in red cell velocity and capillary density. A particularly interesting result was that the flow is also regulated when tissue  $pO_2$  is above the critical level ( $pO_2 = 5\text{mmHg}$ ) at which level it was believed it could not influence metabolism *in vitro* [Jöbsis, 1972]. By studying individual arterioles autoregulatory responses to alterations in arterial or venous perfusion pressure within the rat cremaster muscle at different bath  $pO_2$  value, more pronounced vasodilation due to the change of infusion pressure was found when  $pO_2$  was low. It was concluded that autoregulation dilation of arterioles was reduced but not abolished by elevated tissue oxygen [Morff, 1982]. Stowe *et al* [1975] indicated that tissue  $CO_2$  tension and/or pH are also involved in the autoregulation along with oxygen tension in dog gracilis muscle perfused at constant flow. Davis [1988] further concluded that both oxygen and carbon dioxide tension are key variables in the local control of muscle blood flow and they might account for nearly all of the blood flow response to steady-state exercise in bat wings. However, some other studies could not confirm that muscle blood flow is consistently related to venous oxygen tension [Duling, 1975; Granger, 1976; Sparks, 1980]. It has been suggested that both mechanisms are simultaneously contributing to the local vascular region although Morff [1982] observed vasoconstriction in response to increased venous pressure in the artery of the rat cremaster muscle and suggested that the predominant mechanism was myogenic, but that metabolic mechanisms were also operative in skeletal muscle.

### **2.2.3 Mesenteric Circulation**

In the mesenteric system, the dilation of individual arteries following reduction in arterial pressure could also be interpreted by either the myogenic or metabolic hypotheses [Burrows, 1981]. Elevation of venous pressure could differentiate between these two mechanisms [Johnson, 1964]. Burrows and Johnson [1981] designed an experiment to test the effect of venous pressure elevation on the arterial

bed by calculating circumferential wall tension as an index of a tension-sensitive myogenic mechanism and arteriolar volume flow as an index of metabolic mechanism in the isolated autoperfused cat mesentery. These results demonstrated that although both mechanisms contribute to local blood flow control with increase of intravascular pressure, it seems that the myogenic mechanism is dominant. However Shepherd and co-workers [1977] found that a reduction of blood flow by 35% abolished the constrictor response to increased venous pressure in the intestine. The response is different from that in skeletal muscle in that non-pulsatile perfusion of the intestine decreased both the occurrence and magnitude of the constrictor response [Shepherd, 1977]. In an isolated rat portal vein, the myogenic response to stretching of the vascular wall depended on muscle length and was strongly influenced by variations in the rate of change in length. The dynamic component appeared much more prominent than the static component [Johnson, 1976].

#### **2.2.4 Renal Circulation**

It has been observed that Glomerular Filtration Rate remains almost unchanged over a range of mean arterial blood pressures between 80 to 180mmHg [Conrad, 1984]. The exact value of the lower limits of the renal autoregulatory range are somewhat variable according to the preparation under study. Renal Blood Flow is also maintained nearly constant over a wide range of arterial blood pressures in the rat, man, dog and rabbit [Forster, 1947; Shipley, 1951; Arendshorst, 1975]. The intrinsic renal sensing mechanism that controls the vasoregulatory response is not known but there are three major hypotheses:

- *The renal vessels respond to some index of transmural pressure, ie an increase in transmural pressure due to increasing arterial blood pressure will cause a myogenic reflex vasoconstriction, thus reducing blood flow. This is the myogenic theory.*

- *Changes in renal perfusion pressure produce parallel alterations in interstitial hydraulic pressure and post glomerular vascular resistance [Hinshaw, 1959; 1964]. A rise in perfusion pressure is thought to lead to an increase in interstitial pressure, compression of the peritubular capillaries and intrarenal veins, and a rise in total renal vascular resistance. This is the tissue pressure theory.*
- *It has been suggested that some signal created by a change in blood pressure is sensed by a tubuloglomerular feedback system which controls the level of angiotensin II by modulating renin release by the juxtaglomerular apparatus [Knox, 1974; Navar, 1978]. This is the tubulo-glomerular feedback theory.*

Due to technical problems in measuring 'renal tissue pressure' either by a needle inserted into the renal parenchyma or by renal venous wedge pressure, it is still very difficult to actually determine the true interstitial pressure. This makes a quantitative evaluation of the tissue pressure theory difficult. Ott *et al* [1971] invented a new method to measure tissue pressure by implanting a rigid-walled, multiperforated capsule into the substance of the renal cortex. It was believed that interstitial fluid would accumulate and equilibrium in this capsule and hydraulic pressure in this space could be used as an index of interstitial fluid pressure. Stromberg [1970] observed that the pressure measured with this type of capsule was related to the development of a membrane of hyaluronate and collagen which fills the pores and surrounds the capsule soon after implantation. Thus, the capsule performed as an oncometer and probably does not reflect interstitial fluid hydraulic pressure alone.

### 2.3 CONCLUSION

It has been recognized for many years that vasoregulation is involved in the local control of many organs. There are many theories to interpret this phenomenon, but none of them explains it completely. Due to methodological problems little work has been done in defining myogenic mechanisms. In metabolic dilation more than one chemical acts on the vascular smooth muscle and different organs such as the



hindlimb, kidney, heart, and skeletal muscle have different dominant chemicals. These differences may reflect intrinsic difference in behaviour in organs.

In this chapter, I emphasize the local control of blood flow in non-coronary vessels. Like other organs, the coronary circulation exhibits the local vasoregulatory phenomenon. The first preliminary description of coronary vasoregulation was by Eckel and co-workers [1949]. Now it is well established that coronary blood flow is regulated according to the heart's demand. Detailed discussion of coronary vasoregulation, especially metabolic vasoregulation, follows in the next chapter.

# **CHAPTER 3**

## **LITERATURE REVIEW**

### 3.1 INTRODUCTION

Coronary flow is regulated so that coronary supply meets demand. The metabolic hypothesis is widely believed to be dominant amongst three hypothetic mechanisms – metabolic, myogenic and tissue pressure – for vasoregulation in local control of coronary blood flow (CBF).

Metabolic regulation of CBF refers to the vasoregulation secondary to the changes of myocardial work or oxygen usage, regardless of perfusion pressure. Many metabolites have been proposed as physiological regulators of coronary flow. There is evidence that oxygen, carbon dioxide and adenosine are vasoactive in the coronary circulation. Other chemicals such as prostaglandins, endothelium-derived relaxing factor (EDRF), potassium and hydrogen ions are also related to local control of CBF at one time or another. Unfortunately each of the metabolites satisfies the requirements in some respects and not in others. Thus, almost all the investigators agree that CBF is determined by several factors rather than by a single factor [Belloni, 1979; Feigl, 1983; Berne, 1979].

The term "metabolism" in the local control of blood flow might encompass the metabolic changes taking place in the tissues which lead to the vasoregulation. The term could refer to the formation or breakdown of the vasoactive substance itself or even to the metabolic changes occurring within the vascular smooth muscle cell leading ultimately to contraction or relaxation [Gregg, 1963]. There are three metabolic hypotheses [Berne, 1979; Feigl, 1983; Johnson, 1986]:

- *any change in the coronary inflow causing a change of metabolic status, such as  $pO_2$ ,  $pCO_2$ , pH, which might affect vascular smooth muscle cells directly, in turn causes contraction or dilation in order to recover metabolic status;*
- *any reduction or increase of coronary inflow induces an increase or decrease of vasodilator metabolites which are also responsible for basal*

*vessel tone in the tissue, causing vasodilation or vasoconstriction in order to keep the balance between blood need and blood supply; and*

- *any change in the coronary inflow may induce a build-up of vasoactive metabolites causing vasorelaxation or vasoconstriction for blood supply and demand balance.*

The important question arises as to whether these three theories can be used for all physiological conditions or whether each theory only explains one particular condition. Whether those putative vasoregulators act together or separately in coronary circulation remains to be determined.

In this chapter, I will review metabolic control of coronary circulation, especially the oxygen and adenosine hypotheses. Other chemicals will also be discussed briefly.

## **3.2 OXYGEN**

### **3.2.1 Oxygen Hypothesis**

There is little oxygen reserve remaining in coronary venous blood because the myocardium efficiently extracts oxygen from the coronary supply under normal conditions. If the heart requires more oxygen in special circumstances, *eg* exercise, this extra demand from the coronary artery will be supplied by the augmentation of **CBF**. The hypothesis that the oxygen deficit is strongly related to **CBF** or is the determinant of **CBF** is more than half a century old [Gremels, 1926; Hilton, 1925].

Working with a Starling heart-lung preparation, Hilton [1924] concluded that "the coronary vessels are extremely susceptible to change in the  $O_2$  tension of blood". In the following year, they performed a series of experiments to demonstrate that reduced arterial oxygen tension of coronary blood elicited an increase of **CBF**, probably due to a direct relaxation of the vessel wall [Hilton, 1925]. However, they did not eliminate other metabolites produced by hypoxia because although they found

that perfusion with fresh deoxygenated blood still caused the increase of **CBF**, this could not exclude the release of vasoactive metabolites during that episode [Berne, 1957].

The mechanism responsible for this precise matching between coronary inflow and myocardial oxygen requirements appears to be entirely local in nature. Pharmacologic autonomic nervous system blockades or denervation of the heart do not alter the ability of the coronary blood vessel to regulate [Berne, 1964]. If  $O_2$  is responsible for coronary vasoregulation, it is expected that hypoxia should increase **CBF** and that hyperoxia should decrease **CBF**. In isolated rabbit hearts, by changing the oxygen content of arterial perfusion by diluting hemoglobin at constant perfusion pressure and heart rate, it was found that a decrease in oxyhemoglobin concentration results in an increase of **CBF**, and augmentation of oxyhemoglobin concentration induces a diminution of **CBF**. Unfortunately they were not able to predict the magnitude of change in flow [Guz, 1960]. Breathing 100% oxygen resulted in a decrease of coronary flow in some experiments [Daniell, 1968; Eckenhoff, 1947; Ganz, 1972]. However, interpretations for those experiments were confused by the change of **CBF** accompanying a decrease in heart rate [Ganz, 1972], a decrease in myocardial oxygen consumption ( $\dot{MVO}_2$ ) [Daniell, 1968; Eckenhoff, 1947; Sobol, 1962] and an increase in coronary sinus oxygen content [Daniell, 1968; Sobol, 1962]. Significant diminution in myocardial blood flow during inhalation of 100% oxygen after coronary occlusion was demonstrated by Rivas and co-workers [1980] without significant changes of heart rate, mean arterial pressure, and mean left atrial pressure. This suggests that arterial hyperoxia has a vasoconstricting effect on coronary circulation and the effect is independent of alteration in  $\dot{MVO}_2$ .

### 3.2.2 Can Coronary Sinus $pO_2$ Reflect Myocardial Tissue $pO_2$ ?

It is very common to use coronary sinus (CS) oxygen tension to reflect the myocardial oxygen tension. The question arises that whether the changes of  $p(cs)O_2$

and  $p(t)O_2$  are always coincident, and more specifically, under which conditions CS oxygen tension can be used as an index of tissue oxygenation. The techniques employed to estimate tissue oxygen tension in the past include a carbon monoxide binding method [Coburn, 1973] and a polarographic technique [Moss, 1970] in which platinum electrodes were inserted into the myocardial wall.

The rationale for CS  $pO_2$  to represent tissue  $pO_2$  is based largely on the proposal that significant gas exchange occurs across noncapillary vascular walls [Duling, 1970; Whalen, 1973]. Schmidt and Niesel [1968] had provided evidence to support a close correspondence between tissue and CS  $pO_2$  because both CS  $pO_2$  and superimposed oxyhaemoglobin saturation remained proportional to each other and is a function of the mean saturation of myoglobin in the heart. CS  $pO_2$  and mean polarographically determined myocardial  $p(t)O_2$  are approximately equal in open-chest dogs [Lösse, 1973] and in isolated saline-perfused cat hearts with a perfusion pressure of 78mmHg [Schubert, 1978]. Coburn and co-workers [1973] reported that mean canine myocardial  $p(t)O_2$  in equilibrium with myoglobin is only 4-6mmHg by using a carbon monoxide binding method. The more interesting result was that they found this  $p(t)O_2$  is constant in spite of large changes in arterial oxygen tension above a critical level of 30-35mmHg. Unfortunately, they did not observe the change of CBF under these circumstances even though it implied indirectly that coronary circulation can maintain a constant mean tissue oxygen tension if arterial oxygen tension is above a critical level.

In addition,  $p(t)O_2$  is not uniform throughout the myocardium [Whalen, 1973; Lösse, 1975; Schubert, 1978].  $p(t)O_2$  histograms show 60% of the myocardium has levels lower than CS  $pO_2$  [Lösse, 1975]. When raising perfusion pressure to 113mmHg, CS  $pO_2$  was raised by 20% without significant changes in mean  $p(t)O_2$  and CBF in the isolated saline-perfused cat hearts [Schubert, 1978]. Using a polarographic technique, 100% oxygen inhalation only caused a 7mmHg increase in

intramyocardial oxygen tension without any increase in coronary venous  $O_2$  tension, but a five-fold increase in arterial blood  $O_2$  tension [Moss, 1970]. Thus, sometimes it is dangerous to use CS  $pO_2$  as an indicator of  $p(t)O_2$ . On the other hand, because of  $p(t)O_2$ 's uniformity across myocardium, it is possible that some region in the myocardium beyond the point of measurement of  $p(t)O_2$  may be anoxic or hypoxic. Thus, those hypoxic regions could produce vasoactive metabolites which are responsible for regulation of CBF without noticeable changes in CS  $pO_2$  or measured  $p(t)O_2$  [Belloni, 1979]. It is still unknown whether this uniformity of  $p(t)O_2$  throughout the myocardium is related to some recent findings by Hoffman's laboratory [Austin, 1990], which demonstrated a profound spatial heterogeneity of blood flow reserve in all layers of the canine left ventricle at normal perfusion pressure. It would be valuable to explore their relationship further to determine the role  $p(t)O_2$  plays in local regulation of CBF.

### 3.2.3 Oxygen Characteristics

#### 3.2.3.1 Oxygen Metabolism

Energy carried mainly in the form of adenosine triphosphate (ATP) is used to perform mechanical work in the myocardium. About 90% of ATP is generated by oxidative phosphorylation (aerobic) [Kobayashi, 1979]. CS oxygen levels are about 5ml/100 ml depending on concentration of hemoglobin in the blood [Xhonneux, 1969], and the coronary artery oxygen tension is reasonably constant with changes in myocardial activity under basal conditions, only very little more oxygen can be extracted from arteries when the heart requires more oxygen. In other words, the major increase of oxygen requirement can only be met by an augmentation of CBF to maintain a nearly constant, very high level of arteriovenous oxygen extraction. One direct measurement in the cat heart showed that intracellular  $pO_2$  ranges between 0 and 30mmHg *in situ* [Whalen, 1973]. Moss's [1970] experiment showed that pure oxygen inspiration induced a decrease of CBF, moderate increase in tissue oxygen

tension, but non-alteration in coronary sinus oxygen tension. This suggested that the decreased CBF may elicit more extraction of oxygen in order to supply enough blood to the myocardium.

#### **3.2.3.2 Oxygen Vasoactive Properties**

It is unclear whether oxygen itself has vasoactive properties which are a direct interaction between oxygen and vascular smooth muscle or the changes of vascular tone due to an imbalance between oxygen supply and demand are dependent on the release of some vasoactive substances from the surrounding parenchyma. Gellai [1973] showed that, with isolated helical vascular strips, contractile tension to acetylcholine was not affected or was only 5-20% affected by lowering the  $pO_2$  to 5-10mmHg. When the  $pO_2$  nearly reached 0mmHg, contractile activity was markedly depressed. However, with adenosine in the bath, a decrease in  $pO_2$  from 100 to 10mmHg did influence the effect of adenosine on contractile tension. This suggested that local  $pO_2$  could regulate the vascular changes induced by adenosine and that the equilibrated concentration of release and washout of adenosine in the vicinity of the smooth muscle cells of the small coronary vessels would allow oxygen to exert its direct regulatory effect under normal condition.

#### **3.2.3.3 Mechanism of Vasoactive Action**

Basically there are two mechanisms proposed for oxygen vasoaction:

- (a) *a direct effect of oxygen on vascular smooth muscle and*
- (b) *an indirect effect of oxygen on vessels possibly through carbon dioxide, adenosine or other metabolites; also possibly related to autonomic nerve reflexes.*

Carrier and co-workers [1964] found that the resistance to flow through isolated arterial segments decreases as a function of the oxygen tension (below a critical value) of the perfusing blood, without a change of blood viscosity. A similar



result was demonstrated in isolated strips of rabbit aorta, where the response to epinephrine decreases as a function of the oxygen tension of the bathing fluid [Detar, 1966]. Both authors suggested that oxygen tension has a direct effect on vascular smooth muscle when  $pO_2$  is below a critical value. An experiment was designed to determine contractile responsiveness to hypoxia in isolated aortic and skeletal vessels in various sizes from 200 $\mu$ mD to 20 $\mu$ mD. It was found that hypoxia produced greater depression of contraction when oxygen demand in vascular segments is low than when it is high [Chang, 1980]. This result can not be explained by restricted energy metabolism. This supports the direct action of oxygen on vascular smooth muscle. Numerous studies on strips isolated from vessels have also supported this hypothesis [Detar, 1980; Kalsner, 1977]. However, none of these workers was able to offer direct evidence for the contention that oxygen produces vasoregulation by direct interaction with the smooth muscle cells in the heart. one study [Belloni, 1977] demonstrated that CS oxygen content always changed more quickly than did coronary vascular resistance after correction for vascular transit effects following a step change in heart rate in anaesthetized dogs. They concluded that  $M\dot{V}O_2$  changes more rapidly than does coronary resistance following 20beats/min changes in heart rate. From this it seems logical to think that the delay of coronary resistance variation would allow the mediators related to oxidative metabolism to generate and accumulate to the concentration which could trigger vasodilation. A few candidates related to oxidative metabolism, *eg*  $O_2$ ,  $CO_2$ , adenosine and prostaglandins, have been postulated to be responsible for this vasodilation<sup>1</sup>.

To determine whether oxygen can have a direct influence on vascular smooth muscle, Pittman [1981] posed three key questions:

(a) *What is the normal  $pO_2$  physiological range for resistance vessels?*

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<sup>1</sup>The interaction amongst those candidates will be discussed later.

- (b) *What is the  $pO_2$  critical value for vascular smooth muscle cells to be sensitive to its change?*
- (c) *What is the mechanism for vascular smooth muscle cells to react to changes in their local  $pO_2$ ?*

Since mitochondria are the major sink for oxygen in the cells, studying the relationship between the function of mitochondria and oxygen tension is very important. It has been found that the function of isolated mitochondria is independent of  $O_2$  concentration until very low values are obtained [Sugano, 1974; Oshino, 1974]. The critical value is 1-5mmHg for isolated cells [Wilson, 1979]. The critical  $pO_2$  *in vitro* for mitochondria, which is the local  $pO_2$  below which oxygen becomes unavailable for electron transport and oxidative phosphorylation, is nearly zero [Jöbsis, 1972]. However, Hempel and co-workers [1977] demonstrated that the critical  $pO_2$  for mitochondria in intact tissues could be above zero and may be as high as 50mmHg. One study has shown that substantial intracellular oxygen concentration gradients established by mitochondrial respiration between the suspending medium and the mitochondrial inner membrane occur under hypoxic conditions in isolated cardiac myocytes. It was concluded that this intracellular oxygen gradient is an important factor in determining the function of isolated mitochondria in cells and may contribute to the oxygen dependence of cardiac myocyte function *in vivo* [Jones, 1986].

To study whether the arterial oxygen tension directly controls CBF, Berne *et al* [1957] set up an experiment with special conditions which allowed CS blood rich in oxygen not to fall below 5.9vol%, and arterial blood poor in oxygen tension to be as low as 11.7vol%, by means of high perfusion pressure in open-chest dogs under beating and fibrillating heart conditions. They found that there was no concomitant increase in CBF. On the other hand, at normal perfusion pressure, linear correlation between CS oxygen tension and CBF was found when hypoxemia reached the point at which CS oxygen content was below 5.5 vol% regardless of the arterial oxygen tension. As a result, they concluded that the low arterial oxygen tension could not

itself increase coronary flow, and that since coronary venous oxygen reflected myocardial tissue oxygen content, this latter parameter probably regulates CBF. Cytochrome aa3 was proposed as a vascular oxygen receptor, but poisoning the enzyme with cyanide did not cause as much dilation as hypoxia [Coburn, 1977]. Since the contractile activity of single vascular smooth muscle cells is not effected by oxygen until the  $pO_2$  falls below about 8mmHg [Berne, 1980] which is lower than the  $pO_2$  experienced by resistance vessels *in vivo* under normal conditions, it is unlikely that oxygen will have a direct effect on resistance vessel tone.

The vascular reaction to imbalance in oxygen supply and demand may be attributed to oxygen by way of some other metabolites which are related to oxidative metabolism produced by the parenchymal cells. Berne [1963] found the breakdown products of adenosine in CS blood during cardiac hypoxia. It was suggested that hypoxia has an indirect effect on vascular smooth muscle and adenosine may be responsible for triggering the vasodilation due to hypoxia. Considerably evidence has been published supporting adenosine as a potent physiological vasodilator in coronary circulation<sup>1</sup>. However, there are no substantial experiments firmly supporting oxygen's indirect effect on vascular smooth muscle by releasing adenosine. Thus, we know that oxygen is related to the regulation of CBF, but how they relate to each other remains to be determined.

### **3.2.4 Oxygen and Cardiac Activity**

#### **3.2.4.1 Oxygen and Vascular Basal Tone**

The coronary system has higher basal resistance than the brain or kidney. Myogenic coronary constriction seems the likeliest and most important factor responsible for this. Transmural pressure differential is the major stimulus supporting

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<sup>1</sup>This will be discussed in the adenosine hypothesis section.

myogenic tone. Electrophysiological studies showed myocyte membrane potential was inversely related to the vessel calibre [Harder, 1979]. To develop myocyte membrane potential requires energy. There is no doubt that oxygen plays an important role in the maintenance of coronary vascular basal tone.

#### **3.2.4.2 Oxygen and Increased Cardiac Work**

The foundation for the hypothesis that oxygen is responsible for coronary regulation during increased cardiac work is the observation that the left CBF apparently depends on  $\dot{MVO}_2$  [Katz, 1958; Khouri, 1965]. This correlation could be interpreted in three ways. One is causally related, *ie* increase of cardiac work causes augmentation of oxygen usage which elicits a fall of  $p(t)O_2$ , therefore precapillary sphincters are sensitive to this change and induce vasodilation (direct oxygen effect on the vascular smooth muscle). Another interpretation is covariant, *ie* augmentation of  $\dot{MVO}_2$  (rate of metabolic production) due to increased cardiac work causes release of oxygen-dependent vasoactive metabolite(s) into interstitial spaces and evokes vasodilation (indirect effect of oxygen on the vascular smooth muscle). In this case, the vasoactive metabolite(s) should be proportional to the increased oxygen usage. The last interpretation is that the correlation is only coincidental, *ie* some other concomitant change during increased cardiac activity stimulates production of vasoactive metabolite(s) which causes vasodilation.

#### ***Can $\dot{MVO}_2$ be the regulating factor for CBF?***

$\dot{MVO}_2$  refers to the volume of oxygen consumed by the heart and is determined by the situation and the type of activity the heart is performing. Over the years, many indices have been developed and used as reliable predictors of the oxygen consumption of the heart. Cardiac oxygen consumption is calculated by the product of CBF and artery-venous oxygen content difference under steady-state conditions by

means of the Fick principle. To determine whether  $\dot{MVO}_2$  is a regulating factor for CBF, there are a few questions that need to be answered.

- a) *Does a linear relationship exist between CBF and  $\dot{MVO}_2$ ?*
- b) *Through what mechanism(s) – direct or indirect – does  $\dot{MVO}_2$  control CBF?<sup>1</sup> in vitro in vitro*
- c) *No matter how  $\dot{MVO}_2$  is changed, is it always the same mechanism which regulates CBF?*

Most data on the subject show a fairly linear steady-state relationship between flow and  $\dot{MVO}_2$  [Rubio, 1975]. The correlation coefficients between CBF and  $\dot{MVO}_2$  are typically 0.8 [Eckenhoff, 1947] to 0.9 [Foltz, 1950; Nelson, 1974] under static and dynamic exercise conditions. In such a close relationship, one would expect that the changes of flow are always proportional to the changes of  $\dot{MVO}_2$ . The relationship between  $\dot{MVO}_2$  and CBF was modified by changing certain hemodynamic conditions such as aortic pressure, cardiac output and heart rate [Braunwald, 1958]. When cardiac work and  $\dot{MVO}_2$  were elevated by increasing aortic pressure, the percentage increment in CBF was greater than the percentage increment in  $\dot{MVO}_2$ . In contrast, when work and  $\dot{MVO}_2$  were increased by augmenting cardiac output, the percentage increment in CBF was less than the percentage increment in  $\dot{MVO}_2$ . However, the work done by our laboratory demonstrated that both increased aortic pressure and elevating stroke volume have an equal effect on the regulation of CBF in terms of the same cardiac work [Wright, 1989]. One experiment [Sarnoff, 1963] allowed CBF to change without disturbing other hemodynamic conditions in the isolated dog hearts. Over a wide range of CBF,  $\dot{MVO}_2$  did not change if the measures of the cardiac activity as aortic pressure, heart rate and stroke volume were held constant. However,

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<sup>1</sup>This is to determine whether oxygen has a direct or indirect effect on vascular smooth muscle if myocardial oxygen consumption controls CBF. This has been discussed in section 3.2.3.

when **CBF** were held constant,  $\dot{MVO}_2$  varied in the same direction as the amount of ventricular myocardial tension. This indicated  $\dot{MVO}_2$  could be not changed while the coronary artery flow is regulated. The special phenomenon of reactive hyperemia after artery occlusion in the coronary system was showed to be tightly related to myocardial metabolic activity during the interval of arterial occlusion and is not influenced by alterations in resting **CBF** which occur independently of  $\dot{MVO}_2$  [Bache, 1973]. Thus,  $\dot{MVO}_2$  is at least partially responsible for coronary reactive hyperemia. Part of the variation of **CBF** could not be explained by the level of  $\dot{MVO}_2$ . Bache suggested that this variation could be due to another mechanism which is not directly related to oxidative metabolism.

If one accepts that  $\dot{MVO}_2$  determines **CBF**, one plausible expectation would be that changes in the amount of cardiac work in any condition will result in parallel changes of **CBF**. In cat papillary muscle tension studies, evidence was presented that alteration in shortening and external work at a constant intrinsic velocity were associated with changes in  $\dot{MVO}_2$  [Coleman, 1969]. It has been known for many years that exercise is accompanied by a marked increase in **CBF** [Bassenge, 1973; Vatner, 1972; Van Citters, 1969; Restorff, 1975]. One elegant experiment done by Khouri and co-workers [1965] showed the effect of graded treadmill exercise on heart rate, blood pressure, main left coronary artery flow, stroke coronary flow, cardiac output and stroke volume in intact unanaesthetized dogs. There is close parallelism amongst heart rate, coronary flow and cardiac output. In calculating myocardial oxygen usage by multiplying the coronary arteriovenous difference by the coronary flow, and the left ventricular work by timing the cardiac output with blood pressure, a close correlation between cardiac work, oxygen usage, and coronary flow was found. It was concluded that cardiac work is an important determinant of myocardial oxygen usage and **CBF**. However, other work produced conflicting results: when the frequency of stimulation of the heart and the aortic pressure are kept constant, changes of the work performed do not result in change of **CBF**; also, when the working

conditions of the heart are kept constant, changes of **CBF** do not cause any variation of the amount of work performed [Rosenblueth, 1961]. It should be noted that the cardiac work was estimated by mechanical measurement in their experiment. The important question is whether the work performed by the heart itself determines **CBF** or the change of work results in other changes such as  $\dot{MVO}_2$  or even other metabolic products, the latter having an effect on vascular smooth muscle to elucidate vasoregulation.

### **3.2.5 Conclusion**

Recent evidence indicates that coronary metabolic vasoregulation is closely coupled to oxygen usage by the heart. Hypoxia causes vasodilation and hyperoxia results in vasoconstriction. Oxygen could be responsible for the local control of **CBF** during increased cardiac work. This implies that the metabolic vasoregulation is to keep oxygen stable. However, the evidence in support of this hypothesis is indirect and does not prove myocardial hypoxia is the initiating factor. The mechanism is unknown. Oxygen may have a direct or indirect (involving other vasodilator agents) effect on the vascular smooth muscle. There is no data available to directly measure vessel wall  $pO_2$  during vasoregulation and to demonstrate the measured changes sufficient to interpret the re-adjustment of coronary resistance. The interpretations of experimental results were also clouded by concomitant changes during alteration of oxygen content.

## **3.3 ADENOSINE**

### **3.3.1 Adenosine Hypothesis**

Observations on the close correlation between **CBF** and the metabolic activity of the myocardium had led Berne to study the products of metabolism under hypoxia in 1963. In the isolated cat heart, coronary vasodilation and a reduction in the heart rate were associated with the anoxia-induced appearance of inosine and hypoxanthine

[Berne, 1963]. In the intact working heart of open chest dogs [Jacob, 1960], concentrations of inosine and hypoxanthine in the CS were found to be from 3 to 27 times more during myocardial hypoxia than the concentration determined after doubling CBF by infusing adenosine into the left coronary artery. Neither of the adenine nucleotide derivatives was present in significant amounts in arterial blood of hypoxic dogs or in arterial or cardiac venous blood of the normally ventilated animal, indicating that inosine and hypoxanthine came from the myocardium.

Berne [1963] assumed that the inosine found in the CS is in some measure derived directly from adenosine by deamination and not by the dephosphorylation of inosinic acid. In other words, the degradation of AMP to inosine and hypoxanthine occurs via adenosine rather than via inosine monophosphate (IMP). Based on studies by Drury [1929], who demonstrated that adenosine is a potent vasodilator, and by Jelliffe [1957], who showed that reoxygenated CS blood collected from hearts subjected to hypoxia or ischemia failed to produce changes in vascular resistance when infused into the coronary artery, which indicated that if a vasoactive substance is released from the myocardium during hypoxia it is rapidly inactivated and greatly diluted by the blood. Berne proposed that adenosine acts as a transmitter signalling the oxygen tension at a critical site in the myocardial cell to the coronary vascular smooth muscle cells.

Berne's *adenosine hypothesis* for coronary vasoregulation has generated many further studies. Generally, these studies have been designed to show that adenosine meets the criteria for a compound to be a chemical transmitter responsible for vasoregulation. These criteria are [Berne, 1980]:

- a) *The compound has to be found in the interstitium under certain conditions, and also widely spread throughout the vascular bed.*
- b) *The compound itself should be a potent vasodilator in the physiological quantities found in the interstitium and endogenously infused, and should*



*completely mimic the physiological reaction which has been seen endogenously.*

- c) The time course of production of the compound should be the same as the vasodilation: a precise quantitative cause and effect relationship should be established between the interstitial fluid concentration of the compound and the diameter of the coronary resistance vessels.*
- d) The various inhibitors and blocking agents should have effects consistent with the hypothesis whether the compound is released physiologically or artificially applied.*

### **3.3.2 Adenosine Characteristics**

#### **3.3.2.1 Adenosine Metabolism**

The processes of adenosine metabolism in the heart are shown in Figure 3.1 [Berne, 1979; Feigl, 1983; Schrader, 1990; Lloyd, 1988]. Adenosine is formed by dephosphorylation of AMP. The enzyme, 5'-nucleotidase which catalyzes this reaction, is located in membranes of cardiac cells [Nakatsu, 1972; Rubio, 1973; Nees, 1980]. It can act both inside and outside cardiac cells to convert AMP to adenosine [Frick, 1976; Schütz, 1981], and also as a translocase to move adenosine through the cell wall. Then adenosine can act on resistance vessels to produce vasodilation through either facilitated diffusion into interstitial spaces, or by being formed outside the cardiac cell membrane, which permit it to be in contact with extracellular fluid [Berne, 1979]. However, there is no substantial evidence to support this. The critical point is that 5'-nucleotidase must have sufficient activity and an appropriate location in the myocardium for the formation of adenosine from AMP to prevent cardiac adenylic acid deamination of AMP. Recent views on the formation of adenosine have been modified by the observation that most of the adenosine in isolated well-oxygenated guinea-pig hearts is derived from the transmethylation pathway by hydrolysis of S-adenosylhomocystein (SAH) intracellularly [Lloyd, 1988].

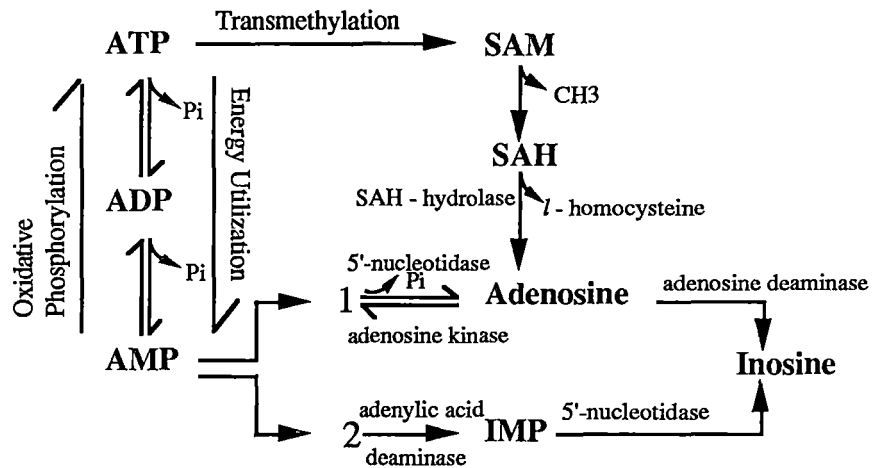


Fig. 3.1: Pathways of adenosine formation (see text for detail)

Two paths for adenosine inactivation are shown in Figure 3.2: either uptake by cells followed by rephosphorylation to **AMP** by adenosine kinase or deamination to inosine catalyzed by adenosine deaminase. Adenosine readily enters cells, *eg* red blood cells [Kolassa, 1978; Roos, 1972], myocardial cells [Cobbin, 1974; Olsson, 1973], leukocytes [Strauss, 1976] and cells in tissue culture [Plagemann, 1974] by 5'-nucleotidase translocation, simple diffusion and carrier-facilitated diffusion [Berlin, 1975; Fox, 1978; Paterson, 1981]. On the other hand, adenosine deaminase has

#### Interstitial Adenosine

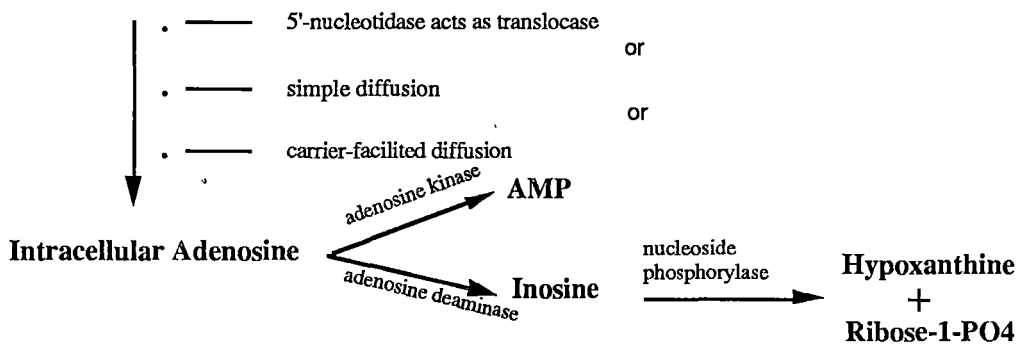


Fig. 3.2: Pathways of adenosine degradation

been found to be ubiquitous in the heart [Baer, 1966]. Adenosine deaminase catalyzes the rapid degradation of adenosine to inosine and purine nucleoside phosphorylase catalyzes the breakdown of inosine to hypoxanthine and ribose-1-PO<sub>4</sub>. Finally hypoxanthine forms xanthine under the influence of xanthine oxidase. Apparently this

is a major problem for detecting adenosine from heart owing to high intracellular activity of adenosine deaminase. Rubio *et al* [1972] found that nucleoside phosphorylase is restricted to the endothelial cells and the pericytes in the heart. They could not detect any nucleoside phosphorylase activity in the cardiac cells by histochemical and electron microscopy techniques. This probably explains the observation that measurements of adenosine concentrations are higher in the myocardium than in the venous effluents of perfused hearts.

It has been demonstrated that 5'-nucleotidase activity is inhibited by ATP [Baer, 1966; Burger, 1967]. Baer and his colleagues postulated that when myocardial hypoxia occurs or cardiac performance increases, the reduction of ATP will diminish the inhibition of 5'-nucleotidase which will lead to increased formation of adenosine. Unfortunately, this attractive hypothesis became invalidated when it was found that ADP is a more potent inhibitor of 5'-nucleotidase than ATP [Edward, 1970; Sullivan, 1971].

#### **3.3.2.2 Adenosine Vasodilating Properties**

Drury [1929] demonstrated that adenosine infused into coronary circulation resulted in coronary vasodilation. This has been confirmed in latter studies in dogs [Essex, 1940] and rabbits [Wedd, 1931; Wolf, 1956]. There is no doubt that exogenous adenosine is a coronary potent vasodilator [Berne, 1979; Feigl, 1983].

#### **3.3.2.3 Mechanism of Vasodilating Action**

Although some work has been carried out to determine the mechanism(s) of adenosine's vasodilating action and a few theories have been postulated, the results are by no means conclusive. Almost all the work has been performed by means of exogenous adenosine owing to its very short life and high permeability. Among those putative mechanisms, the most attractive is the extracellular adenosine receptor hypothesis.

In an attempt to determine where adenosine acts in the coronary vessels, a synthesized adenosine derivative linked with oxidized stachyose – a high molecular weight protein – was used by Olsson [1976]. This compound has been shown to be too large to penetrate cell membranes. Intracoronary infusion of this linked adenosine compound elicited dose-dependent coronary vasodilation [Olsson 1976]. In the isolated perfused heart, Schrader [1977] reported that a protein-AMP-conjugate which is produced by coupling adenosine with 5'-AMP via phosphoamide linkages to a large molecular weight protein, demonstrated the same potent coronary vasodilation reaction as adenosine and AMP. Both experiments indicate that the adenosine receptor is most likely to be located at the surface of the vascular smooth muscle cells.

Establishing the location of the adenosine receptor has not answered the question "How does adenosine actually trigger the vasodilation action?" Two mechanisms have been proposed, in one smooth muscle cyclic 3',5'-AMP (cAMP) is increased and in the other the inward calcium current during the action potential in vascular smooth muscle is inhibited [Berne, 1979; Feigl, 1983]. In Berne's laboratory, effects of adenosine on calcium influx [Harder, 1979; Herlihy, 1976] have been studied in isolated large and small coronary arteries. It has been shown that adenosine abolished the action potentials in vascular smooth muscle and this action could be rapidly recovered totally or partially by addition of adenosine deaminase or excess calcium, but there was no direct measurement of calcium flux after adenosine administration. This finding is in agreement with Schnaar and Sparks [1972], who observed adenosine-elicited relaxation of dog coronary arteries contracted with 35mM  $K^+$  and suggested that adenosine acted by decreasing the membrane permeability to  $Ca^{2+}$ . However, Verhaeghe's [1977] experimental result was not consistent with those of Schnaar and Sparks as he found that adenosine inhibited the noradrenaline contraction of canine saphenous vein equilibrated in  $Ca^{2+}$ -free solutions. It is still unknown how adenosine inhibits the calcium uptake in vascular smooth muscle cells –

by its effect on adenosine receptors or by other mechanisms which change membrane conditions. Based on the observations:

- *Adenosine increased cyclic AMP levels of guinea pig cerebral cortex slices* [Sattin, 1970];
- *Exogenous adenosine produced coronary dilation and an increase in cyclic AMP release* [Huynh-Thu, 1978] and
- *A good correlation was shown between cyclic AMP concentration and adenosine relaxation of isolated coronary vascular strips* [Kukovetz, 1978],

it was proposed that adenosine induces coronary vasodilation by increasing vascular smooth muscle cyclic 3',5'-AMP concentration [Berne, 1979; Feigl, 1983]. However those observations require a very high adenosine concentration – about 100 times higher than in physiological coronary vasodilation.  $10^{-4}$ M adenosine failed to produce augmentation of cAMP [Herlihy, 1976]. Veahghe [1977] also observed no change in the cAMP content when the canine saphenous vein was exposed to adenosine. After inhibiting the enzyme phosphodiesterase which degrades cAMP, the adenosine relaxing effect was enhanced on isolated coronary vascular strips [Napoli, 1980]. Therefore, it would be unlikely for adenosine-elicited vasodilation to be due to increments in intracellular cAMP. Unfortunately these findings were not sufficient to decide whether the adenosine-induced potentiating of the stimulated adenylate cyclase was produced by an interaction with the binding of the hormones to their respective receptor sites, or by an interference with the catalytic unit of the enzyme.

Raberger and co-workers [1970] investigated the effects of intracoronary adenosine infusion on myocardial metabolism. This results revealed an increase in myocardial glucose uptake, myocardial lipolysis and lactate and hydrogen ion release. They was concluded that adenosine infusion produces coronary vasodilation via

acidosis and increased carbon dioxide production, both contributing to adenosine metabolic effects enhancing glycolysis. Further studies to test this hypothesis demonstrated that [Raberger, 1971]:

- *exogenous adenosine became a more potent coronary vasodilator in the acidosis and hypercapnia environment and*
- *adenosine stimulated increased glucose uptake was secondary to increased coronary blood flow instead of an insulin-like action .*

Other studies showed that the increase of carbon dioxide after adenosine vasodilation was due to a flow-dependent washout in isolated cat hearts [Turnheim, 1976]. In another set of experiments, adenosine infusion and acidosis were found to relax vascular smooth muscle by a different mechanism [Turnheim 1977]. This did not support Raberger's hypothesis.

#### **3.3.2.4 Adenosine Inhibitors**

It is important to study inhibitory agents for adenosine in order to understand biological phenomena or to assess their physiological significance. Two groups of chemicals have been found to be effective blocking agents: xanthine derivatives and adenosine deaminase.

##### **1.Xanthine Derivatives**

Theophylline and aminophylline are the most common xanthine derivatives used for studying coronary vasoregulation. Aminophylline is a more soluble form of theophylline and contains ethylene diamine.

In anaesthetized animal preparations, aminophylline elicited coronary vasodilation when administered either intravenously [Gilbert, 1929; Oei, 1977] or directly into coronary beds [Boyer, 1941; Melville, 1950]. However, in intact and conscious dogs, intravenously injected aminophylline produced coronary constriction

even though there were corresponding increases in myocardial contractivity and arterial pressure which were expected to result in metabolically mediated coronary vasodilation [Rutherford, 1981]. This vasoconstriction was inhibited by  $\alpha$ -adrenergic blockade, indicating that this effect may be sympathetically mediated. In summary, a different effect of aminophylline appears in anaesthetized animals on the one hand and in intact animals and humans on the other. How this occurs remains a question. Some workers demonstrated positive inotropic effects of aminophylline in isolated hearts [Massingham, 1972; Verna, 1976] and increased cardiac rates [Rutherford, 1981]. These effects were produced at high dosages which make it more difficult to adjust for this drug's effect on vascular smooth muscle.

Afonso and co-workers [1970b] demonstrated that aminophylline blocked 50-75% of the coronary vasodilating effects produced by exogenous adenosine administration intravenously or into the coronary artery. The result was consistent with other work which showed that pretreatment with aminophylline or theophylline antagonized the coronary vasodilating effects of injected adenosine in intact hearts [Schütz, 1977; Gross, 1976; Bünger, 1975; Merrill, 1978] and in isolated coronary arteries [Jones, 1981]. This inhibition was not complete.

Recently, it has been reported that 8-phenyltheophylline (**8-PT**), a xanthine analogue, is the most potent available adenosine antagonist while having relatively low activity as an inhibitor of calcium-dependent phosphodiesterase. This independent activity will cause the increases in heart rate, cardiac contractility and  $\dot{MVO}_2$  [Smellie, 1979]. Griffith and co-workers [1981] showed that **8-PT** was 100 times as effective as aminophylline as an adenosine antagonist in isolated vascular ring segments of rabbit basilar arteries. Bache *et al* [1988] showed that **8-PT** elicited  $95 \pm 1.2\%$  inhibition of the excess flow volume in response to  $20 \mu\text{g}$  adenosine intracoronary infusion, while adenosine deaminase only produced  $73 \pm 11\%$  inhibition, without causing significant alterations of cardiac activity. Thus **8-PT** is the most potent

known adenosine competitive inhibitor and its adenosine antagonistic activity is relatively selective. This will make it the preferred P<sub>1</sub>-purinoceptor antagonist to theophylline or aminophylline.

It has been suggested that this adenosine inhibitory action of xanthine derivatives might occur at the coronary vascular smooth muscle or at the myocardial level [Afonso, 1970a]. Dutta and co-workers [1979] found that methylxanthines compete with adenosine for adenosine binding sites in cardiac microsomal preparations. The antagonism of the inhibitory responses to adenosine by xanthine derivatives such as 8-PT, theophylline and aminophylline was reversed by washing [Griffith, 1981]. When theophylline was linked to stachyose molecules, It was still active in blocking exogenous adenosine effects. This indicates that theophylline acts on receptors on the surface of vascular smooth muscle, but does not establish that theophylline blocks the vasodilatory action of adenosine by competing for a common receptor [Olsson, 1976].

## 2. Adenosine Deaminase

Adenosine deaminase is an enzyme which catalyzes the rapid degradation of adenosine to inosine. Intracoronary infusion of adenosine deaminase has been used to test the adenosine hypothesis in various conditions such as changing coronary perfusion pressure [Dole, 1985], treadmill exercise in conscious dogs [Bache, 1988], and coronary occlusions [Saito, 1981]. By detecting <sup>131</sup>I-labeled adenosine deaminase in cardiac lymph nodes, the presence of the enzyme in interstitial fluid was confirmed [Saito, 1981; Dole, 1985]. During the enzyme intracoronary infusion, Dole *et al* demonstrated that the average value of lymph <sup>131</sup>I-adenosine deaminase activity was  $3.2 \pm 0.4$   $\mu$ /ml which was believed to be able to degrade 29 $\mu$ M adenosine in the interstitial spaces [Dole, 1985]. This indicates that there was more than enough adenosine deaminase penetrating into the interstitium because there was no evidence that cardiac interstitial adenosine concentration ever attained the 29 $\mu$ M level. The



enzyme was also efficacious because it inhibited  $73\pm 11\%$  of coronary vasodilator response to exogenous adenosine [Bache, 1988]. In summary, exogenous adenosine deaminase can be used as an endogenous adenosine blocking agent to study the adenosine hypothesis.

### **3.3.3 Adenosine and Cardiac Activity**

#### **3.3.3.1 Adenosine and Vascular Basal Tone**

Multiple agents maintain basal vascular tone. To verify the hypothesis that adenosine is responsible for the physiological regulation of CBF, many investigators have tried to test whether there is a continuous release of adenosine under normal conditions from myocardial cells into interstitial fluid, and whether lowering the concentration would decrease CBF unless some other mechanism has compensated this decreased flow due to less adenosine [Rubio, 1969; Gewirtz, 1986; Kroll, 1985; Richman, 1964]. Richman [1964] used 8-azaguanine which is considered as an adenosine deaminase inhibitor in a Langendoff-perfused rabbit heart. No adenosine was detected in the venous perfusate under normal oxygenation with or without adenosine deaminase inhibitor. However, adenosine was found in the hypoxic heart with the inhibitor, but not without the inhibitor, indicating under severe hypoxia adenosine was released by myocardial cells into the interstitium. The same result was found by Katori [1966] in guinea pig and cat hearts. However, adenosine was first found in the pericardial fluid of presumably well-oxygenated dog hearts, suggesting a release of adenosine by the heart under normal 'resting' conditions [Rubio, 1969]. It was also found that artificial hypoxia increased the adenosine concentration significantly compared with the well-oxygenated heart. It was concluded that adenosine concentration in the interstitial space regulates CBF to maintain the oxygen balance in the myocardium [Rubio, 1969]. Adenosine was also assayed from 'normal' hearts in pericardial perfusate from both open chest [Miller, 1979] and closed chest dogs [Watkinson, 1979].

Saito *et al* [1981] did not observe a change of CBF during adenosine deaminase infusion under resting conditions, concluding that the nucleoside may not function in a similar capacity under basal, steady-state conditions. Using closed chest, sedated domestic swine, Gewirtz and co-workers [1986] set up a more careful experiment in which adenosine deaminase was infused into the coronary circulation at a dose sufficient to deaminate several times more than the maximal myocardial output of adenosine after having verified that the enzyme penetrates into the interstitial fluid. They failed to demonstrate a decline in regional myocardial flow in each layer in response to adenosine deaminase infusion, but they did observe a significant reduction versus control in the distal-to-circumflex flow ratio for epicardial and transmural layers. The results suggest that adenosine only plays a modest role in maintaining coronary arteriolar tone under steady-state conditions.

After confirming that [Kroll, 1985]:

- a) *the deposited adenosine deaminase was sufficient to deaminate adenosine by showing that the vasodilation induced by adenosine infusion was inhibited in the adenosine deaminase - treated area versus the control region ;*
- b) *the inhibition of adenosine vasodilation was fully recovered with EHNA (an inhibitor of adenosine deaminase ), indicating that the inhibition effect was contributed to the enzymatic action of adenosine deaminase; and*
- c) *adenosine deaminase had a significant effect on interstitial adenosine by showing that deaminase activity was found in cardiac lymph, suggesting its penetration into the interstitial space.*

Kroll and Feigl demonstrated that simultaneous CBF measured with radioactive microspheres was not different in a region treated with adenosine deaminase from a region without adenosine deaminase. Based on this evidence, they concluded that adenosine is normally below the vasoactive threshold and is not an important regulator

for CBF under unstressed conditions. It seems generally agreed that adenosine does not contribute to maintenance of coronary vasomotor tone in basal condition.

### 3.3.3.2 Adenosine and Increased Cardiac Work

Exercise makes CBF increase. If adenosine is responsible for this physiological change, it would be expected that the adenosine concentration in interstitial fluid would increase during exercise. High correlations between the adenosine concentration in pericardial perfusate, which was recognised as reflecting the extracellular fluid adenosine concentration, and  $\dot{M}V\text{O}_2$  as well as coronary vascular resistance were observed during stellate ganglion stimulation in anaesthetized dogs [Watkinson, 1976; Miller, 1979], atrial pacing in anaesthetized dogs [Knabb, 1983; Saito, 1980; Randall, 1985], aortic constriction [Saito, 1980; McKenzie, 1980], isoproterenol infusion [McKenzie, 1980], intracoronary norepinephrine [Manfredi, 1982; Jone, 1982] and during treadmill exercise in conscious dogs [Watkinson, 1977; McKenzie, 1982; Ely, 1983]. Watkinson *et al* [1979] measured the pericardial fluid adenosine concentration in four dogs. Adenosine concentration increased from an average value of  $95.5 \pm 7.6 \text{ pmol/ml}$  at rest to  $263 \pm 19.1 \text{ pmol/ml}$  at a heart rate of  $204 \pm \text{bpm}$ . In isolated saline perfused guinea pig hearts, a correlation between CBF and tissue adenosine production was demonstrated under the influence of several positive and negative inotropic agents [Degenring, 1976]. It was also found an increase in cardiac lymph adenosine concentration – another index of the extracellular fluid adenosine concentration – in response to an increase in cardiac work [McKenzie, 1983]. This was supported by Foley [1978], who observed that pericardial fluid adenosine levels rose with aortic constriction in rats. CBF was not measured. Saito [1980] has suggested that adenosine is a single vasoactive metabolite which is predominantly responsible for the coronary functional hyperemia based on the observation of the consistent parallelism between CBF and  $\dot{M}V\text{O}_2$  under a variety of conditions in which cardiac performance was changed in either direction. Manfredi

and Sparks [1982] reported that vasodilation produced by intracoronary infusion of norepinephrine instead of atrial pacing was accompanied by an increased adenosine release by the heart. The bulk of the data has shown a significant correlation between endogenous adenosine concentration and CBF.

By using positive inotropic agents such as histamine, nor-adrenaline (NA) and adrenaline (A) to stimulate isolated, saline perfused guinea pig hearts, adenosine release by these hearts was correlated with  $\dot{M}V\text{O}_2$  and presumably CBF. CS oxygen content decreased with stimulation. However the change of heart rate (chronotropic agent) by itself has no effect on adenosine release [Wiedmeier, 1977]. Similar results were reported by Saito *et al* [1980]. It was concluded that oxygen may be a potent stimulus for adenosine production. In other words, increased cardiac work produces hypoxia respectively which triggers adenosine to be released into the interstitial space to dilate arteries. However, McKenzie and co-workers [1982] demonstrated that when four factors indicated no myocardial hypoxia (low myocardial lactate content, continued lactate uptake, lack of changes in the lactate-pyruvate ratio, and stable CS oxygen tension), significant correlations ( $r=0.74-0.83$ ) amongst myocardial adenosine content, CS adenosine concentration, and coronary resistance were obtained using conscious treadmill exercising dogs. They suggest that adenosine is responsible for increased CBF during increased metabolic activity, but hypoxia is not the stimulus for adenosine production in treadmill exercise.

More recently interest has been directed toward the role of adenosine in coronary vasodilation during exercise by using an adenosine blocker, either adenosine deaminase by intracoronary infusion or a potent adenosine competitive inhibitor used systemically. Randall and Jones [1985] showed aminophylline intravenous injection attenuated the increase in CBF ( $p<0.05$ ) and enhanced the increase in myocardial oxygen extraction ( $p<0.05$ ) when increasing cardiac rates by pacing. This supports adenosine's role in coronary active hyperemia induced by an increase in heart rates.

However both theophylline [McKenzie, 1987] and aminophylline [Jones, 1982] failed to alter the increase of CBF by isoproterenol administration [McKenzie, 1987] or intracoronary infusion of norepinephrine [Jones, 1982]. The reason for the very discrepant result shown between these two groups of investigators, *ie* an increase of adenosine concentration was obtained after aminophylline administration by McKenzie, but not by Jones after theophylline infusion, is not clear. McKenzie [1987] proposed that adenosine blockade elicited an increase of myocardial adenosine production which may just compensate the competitive inhibition produced by the blockade. Unfortunately, these investigators did not measure coronary sinus  $pO_2$ ,  $pCO_2$ , and pH in order to test whether other putative vasodilators were involved in this reaction. After being sure that both adenosine deaminase and 8-PT produced inhibition of coronary vasodilation in response to exogenous adenosine, Bache [1988] found: a) neither adenosine deaminase nor 8-PT significantly altered coronary vascular resistance and myocardial oxygen extraction; b) neither of them affected CBF, coronary vascular resistance during exercise and the relationship between  $\dot{M}VO_2$  CBF; c) neither of them decrease coronary venous oxygen tension during exercise. These data suggested that adenosine does not play an important role in coronary vasodilation during exercise. It is possible that those previous results imply that adenosine is an important factor for coronary functional hyperemia when oxygen demand is increased by a chronotropic stimulus (*eg* increasing heart rate) and is not an important regulator when oxygen demand is increased by an inotropic stimulus (*eg* isoproterenol, norepinephrine, or systemic exercise), even though these stimuli also have a small chronotropic effect. However, those data without using adenosine blockade showed the opposite result. Current conflicting data provide no answer to this question.

In summary, the coronary vasodilation in response to exogenous adenosine can be nearly completely blocked by an adenosine antagonist (theophylline, aminophylline or 8-phenyltheophylline), but the vasodilation in response to proposed

endogenous adenosine was not or very little affected by those inhibitors during increased cardiac work. Thus, whether adenosine is a mediator responsible for metabolic vasodilation is still an enigma.

#### **3.3.4 Adenosine and Other Vasoregulatory Situations**

Even though this thesis is mainly concerned with active hyperemia in coronary circulation, I will discuss briefly other vasoregulatory situations relating to local control of coronary flow.

##### **3.3.4.1 Adenosine and Reactive Hyperemia**

Reactive hyperemia is the excessively increased flow volume after the release of an arterial occlusion. If adenosine is an essential endogenous vasoregulatory mediator following the occlusion of the coronary artery, the adenosine concentration should reach a certain level which can be responsible for the increase of CBF, and the time course of adenosine production and its levels should be similar to that of the response of reactive hyperemia [Feigl, 1983; Olsson, 1975; Belloni, 1979].

Adenosine appears in CS blood during reactive hyperemia in quantities relating to the duration of the occlusion interval [Rubio, 1969]. In dog hearts, Olsson *et al* detected a marked increase in myocardial tissue levels of adenosine, doubling after 5secs [Olsson, 1970] and 5-6 fold after 15secs [Olsson, 1978] of coronary artery occlusion. During the reactive hyperemic response after coronary occlusion, myocardial adenosine concentrations dropped exponentially in concordance with the flow rate to the control value [Olsson, 1978]. In the isolated guinea pig heart the release of  $^{14}\text{C}$ -adenosine increased and closely paralleled the changes in coronary resistance during reactive hyperemia [Schrader, 1977]. In all cases preocclusion values for the release of labeled adenosine were reached when coronary flow returned to control values after coronary occlusion. This supports the adenosine hypothesis. However, the relationship between coronary flow and myocardial adenosine

concentration was not consistent with the relationship between coronary flow and coronary plasma adenosine levels during intracoronary infusion of adenosine [Olsson, 1978].

Coronary occlusion for very short durations such as one heart beat or even a fraction of diastole, induced no detectable change on myocardial metabolism, but it still elicited a hyperemic response [Eikens, 1974; Giles, 1977; Schwartz, 1982]. Giles interpreted this as myogenic in origin because he believed the period of occlusion was too short to provoke the release of metabolites; in contrast, Shwartz favoured a metabolic mechanism. The key argument is how rapidly coronary occlusion induces the hypoxic stimulus to produce vasoactive metabolites. If myoglobin is completely saturated, the oxygen reserve could support cardiac activity for about 6 seconds in the conscious dog [Olsson, 1964]. It has been observed that cardiac muscle adenosine levels are already substantially increased following 5secs of coronary occlusion [Olsson, 1970], but it still unknown when adenosine is elevated.

By using an intracoronary infusion of adenosine deaminase to destroy adenosine in the cardiac interstitium, the hyperemic response to coronary occlusion was reduced, but not completely abolished [Saito, 1981]. Doubling the rate of adenosine deaminase did not further reduce the intensity of reactive hyperemia. However, we still cannot exclude the possibility that either the adenosine concentration or the adenosine induced vasoactivity was enhanced due to adenosine deaminase administration. These results showed that both theophylline and adenosine deaminase demonstrated the same extent of inhibitory effect in reactive hyperemic response – about 35% reduction. Saito [1981] suggested that adenosine contributes 35% responsibility for this response.

### 3.3.4.2 Adenosine and Autoregulation

The argument for adenosine to be the metabolite responsible for coronary autoregulation is that the change of perfusion pressure would result in change of adenosine production. *ie* increased perfusion pressure induces decreased adenosine release which reduces flow rate under that pressure so that the flow is reasonably constant and vice versa. There have been only three studies addressing adenosine's role in coronary autoregulation [Schrader, 1977; Dole, 1985; Hanley, 1986].

In the isolated guinea pig hearts, a decrease in perfusion pressure in steps from 60-20cmH<sub>2</sub>O which was believed to be the autoregulatory range results in a progressive increase in the rate of adenosine release and in the perfusate concentration of adenosine. The change of adenosine concentration closely paralleled the change of coronary resistance [Schrader, 1977]. This finding supports adenosine involvement in the adjustment of CBF during autoregulation. However, by means of an epicardial well technique, Hanley and co-workers [1986] observed that cardiac interstitial adenosine concentration remained constant during autoregulation although there was a significant difference in coronary resistance, and the coronary bed still autoregulated normally when interstitial adenosine was reduced to levels close to zero by adenosine deaminase infusion in blood perfused dogs *in vivo*. It was concluded that adenosine is not an essential mediator in coronary autoregulation unless the change of adenosine was beyond the sensitivity of the method of detection [Hanley, 1986]. A similar conclusion was made by Dole *et al* [1985] because adenosine deaminase did not alter the time course for coronary pressure-flow relationship either within the autoregulatory range or beyond it. The diametrically conclusions of Schrader and others remain unresolved. As Schrader [1977] has pointed out, a correlation between adenosine and autoregulation can only suggest, but can not prove, that adenosine is the fundamental determinant of changes in coronary resistance during autoregulation.



### **3.3.5 Conclusion**

Although there is much impressive data to support an important role for adenosine in local control of CBF, the adenosine hypothesis remains a hypothesis. Whether the measured adenosine during various cardiac activities is causally related to CBF or just coincidentally related, in other words adenosine is only one form of waste from metabolism, can not be decided with current evidence. Current data appear to suggest that negative data are more convincing than positive ones. It is conceivable that adenosine is not responsible for coronary basal tone, but it can not be excluded as the agent for coronary regulation during exercise. However, a quantitative study of the role of adenosine in reactive hyperemia suggests that adenosine is responsible for a portion of reactive hyperemia. If adenosine is involved in coronary vasoregulation, there are still not sufficient data to determine by what mechanism adenosine triggers vasodilation and whether adenosine is the final agent for regulation of vascular resistance is still unclear.

## **3.4 OTHER PUTATIVE VASODILATORS**

### **3.4.1 Carbon Dioxide and Hydrogen**

A close correlation between cardiac carbon dioxide level and CBF was first demonstrated by Barcroft and Dixon [1907] in the isolated canine heart-lung preparation. It has been repeatedly observed that hypercapnia by inhalation of carbon dioxide gas increases CBF using different experimental preparations [Gremels, 1926; Raberger, 1975; Neill, 1975] and different flow measurements. During constant-flow perfusion, hypercapnia elicited decreased coronary resistance [Case, 1976; Clancy, 1975; Daugherty, 1967]. It has also been demonstrated that hypocapnia caused by hyperventilation decreased CBF [Kittle, 1965; Alexander, 1976]. During constant flow perfusion in coronary circulation hypocapnia increased coronary resistance [Case, 1976; Daugherty, 1967]. However, in other work hypercapnia failed to

induce CBF increases [Kosche, 1971]. The majority of reports states that carbon dioxide is a coronary vasodilator. One group of investigators has gone so far as to suggest that myocardial  $p\text{CO}_2$  is the primary agent controlling coronary flow [Case, 1976]. Those conflicting reports were partially due to differences in the animal preparation and durations of change in  $p\text{CO}_2$  when cardiac hemodynamics were measured [Feigl, 1983].

The aerobic metabolism of myocardial cells generates carbon dioxide continuously as the result of complete oxidative reactions of the substrates. The amount of  $\text{CO}_2$  produced by the myocardium is in proportion to  $\text{M}\dot{\text{V}}\text{O}_2$  determined by the respiratory quotient.  $\text{CO}_2$  is carried in the blood in three forms (as dissolved, bicarbonate and carbamino compounds) and is mainly excreted by the lungs and kidneys.  $\text{CO}_2$  plays an important role in maintaining body pH. It has been well established that  $\text{CO}_2$  and acidosis cause a direct myocardial depressant effect on isolated papillary muscles [Gonzalez, 1971; Mattiazzi, 1977] and isolated perfused hearts [Williamson, 1975]. In the myocardium,  $\text{CO}_2$  has no difficulty reaching the coronary resistance vessels due to its high diffusibility.

Severinghaus *et al* [1967] found that arterial  $p\text{CO}_2$  rather than tissue or venous  $p\text{CO}_2$  correlated with cerebral vascular resistance. Based on the observation that when  $\text{CO}_2$  was blown directly over exposed brain cortex, cerebral vasodilation occurs [Meyer, 1964], it was suggested that  $\text{CO}_2$  acts directly on the smooth muscle cells of arteriolar and arterial vessel walls. This direct relaxing effect on vascular smooth muscle was also shown by others [McLellan, 1974; Radawski, 1972]. After administration of atropine and propranolol or bretylium, the effect of hypercapnia on myocardial blood flow was not altered even though the calculated  $\text{M}\dot{\text{V}}\text{O}_2$  was reduced and the aortic pressure and heart rate were unchanged. Apparently the flow in this situation was over-perfused as far as oxygen demand was concerned. This suggests that elevated  $p\text{CO}_2$  has a direct vasodilating effect on the myocardial circulation instead

of through changes in cardiac work or through the release of autonomic transmitters [Ledingham, 1970]. Unfortunately, those workers did not measure the antagonizing effect of blocking agents. However, in Kontos and co-workers' [1977] studies, they demonstrated that the effect of CO<sub>2</sub> on cerebral blood flow was mediated by local alteration of H<sup>+</sup> concentration. During hypercapnia there is a significant rise in blood epinephrine, norepinephrine, and 17-OH corticosteroids in normal humans [Sechzer, 1960], and increased rate of catecholamine synthesis in rats [Nahas, 1968]. With stabilized  $\dot{M}\dot{V}O_2$  and an adrenergic beta-receptor blockade, sotalol (in sufficient doses to block the effects of 5µg of adrenaline), hypercapnia (arterial pCO<sub>2</sub>=85 mmHg) by CO<sub>2</sub> inspiration failed to increase CBF significantly compared with the effect of hypercapnia in the absence of beta-blockade, but elevated CS pO<sub>2</sub> [Van den Bos, 1979]. It was concluded that high arterial CO<sub>2</sub> causes coronary vasodilation through an adrenergic effect instead of through the direct effect of carbon dioxide.

Rooke and Sparks [1980] examined whether arterial CO<sub>2</sub> mediates a significant fraction of the steady state in CBF while varying  $\dot{M}\dot{V}O_2$  with catecholamine infusions, *ie* how CO<sub>2</sub> influences the relationship between CBF and  $\dot{M}\dot{V}O_2$  during increased cardiac activity. An increased arterial pCO<sub>2</sub> systemically produced a small increase in CBF independent of  $\dot{M}\dot{V}O_2$ . This vasodilating effect was much smaller than the augmentation in flow elicited by increasing  $\dot{M}\dot{V}O_2$ . Thus, the vasodilator potency of CO<sub>2</sub> is very low. They concluded that arteriolar wall pCO<sub>2</sub> is not a major cause of functional hyperemia and suggested that the phenomenon of pCO<sub>2</sub> increases causing an increase in cardiac venous pO<sub>2</sub> at any value of  $\dot{M}\dot{V}O_2$  was due to *Bohr* shift.

As arterial carbon dioxide increases, corresponding decreases in pH (*Bohr's* effect) was expected. In determining whether the effect of CO<sub>2</sub> on CBF is due to the change of pH, Kittle *et al* [1965] demonstrated that increased arterial CO<sub>2</sub> still

augmented **CBF** under constant arterial pH, controlled by the intravenous infusion of 0.9N trishydroxymethylaminomethane.

In summary, many studies have shown that hypercapnia increases and hypocapnia decreases **CBF** when arterial  $p\text{CO}_2$  was the independent variable. If the hypothesis that  $\text{CO}_2$  is a vasodilator responsible for coronary metabolic vasodilation is correct, one would expect that the change of myocardial carbon dioxide in either direction will elicit concomitant changes in **CBF** when other metabolic related substances are constant. Unfortunately, under normal conditions it is not possible to separate the effect of  $\text{CO}_2$  and  $\text{M}\dot{\text{V}}\text{O}_2$  on coronary circulation, because any change in  $\text{M}\dot{\text{V}}\text{O}_2$  will be matched by corresponding change in  $\text{CO}_2$  production under a stable respiratory quotient. In other word, with current techniques it is almost not possible to study the effect of carbon dioxide without changing other metabolic parameters. Whether the changes in **CBF** after the alteration of  $\text{CO}_2$  are caused by the change in pH alone or by the combination of other changes of vasoactive substances, or by the combination of increased  $p\text{CO}_2$  and elevated catecholamines, needs further investigation. There is no evidence for the relationship between the **CBF** and the concentration of myocardial endogenous  $\text{CO}_2$ . There is also not sufficient evidence to show how  $\text{CO}_2$  might alter the contractile state of vascular smooth muscle.

### 3.4.2 EDRF

Furchgott and Zawadzki [1980] were the first to discover that the basal relaxation induced by acetylcholine (**Ach**) and other agonists of muscarinic receptors in isolated rabbit aorta or other arteries was dependent on the presence of endothelium and this endothelium-dependent relaxation by **Ach** results from the release of a non-prostanoid diffusible relaxing factor, called endothelium-derived relaxing factor (**EDRF**) [Furchgott, 1983]. It is generally agreed that **EDRF** is a potent vasodilator substance released by the endothelium [Furchgott, 1980; 1983; Vanhoutte, 1986]. Evidence came from experiments with isolated arteries [Busse, 1985; Furchgott, 1983;

Peach, 1985], large conduit arteries in vivo [Angus, 1983; Pohl, 1986] and resistance-sized vessels in intact rabbit hindlimbs [Pohl, 1987].

**EDRF** half-life values of about 6seconds were reported from a perfusion bioassay system independently for **EDRF** released from rabbit thoracic aorta by **Ach** [Griffith, 1984], for **EDRF** released from cultured bovine aortic endothelial cells by bradykinin [Cocks, 1985] and for **EDRF** released from canine femoral arteries by **Ach** [Rubanyi, 1985]. However, others reported that the half-lives were 25seconds in rabbit aorta and 50seconds in canine femoral arteries [Forstermann, 1986]. This discrepancy was explained by the finding that superoxide dismutase (**SOD**) markedly stabilized **EDRF** released by **Ach** [Rubanyi, 1986] and by bradykinin [Gryglewski, 1986], indicating that superoxide anions ( $O_2^-$ ) can inactivate **EDRF**. Thus, different preparations and laboratories would give different  $O_2^-$  levels. After a number of vasodilators were discovered to be endothelium-dependent relaxing agents (*eg* **A23187**, **ATP**, and **substance P**), it was suggested that extracellular calcium ions play an important role in the release of **EDRF** [Furchgott, 1981; Singer, 1982; Long, 1985]. At an **EDRF** symposium in 1986, Furchgott proposed that **EDRF** is the free radical nitric oxide (**NO**) based on his findings that the characteristics of **NO**-induced relaxation are similar to those of **EDRF**-induced relaxation [Furchgott, 1988]. Palmer [1987] successfully showed that the amounts of **NO** released by bradykinin from cultured porcine endothelial cells were sufficient to account for the biological activity of the **EDRF**.

Based on the findings that nitrovasodilators activate soluble guanylate cyclase of vascular smooth muscle via nitric oxide, and that guanylate cyclase might cause an increase of cyclic **GMP** [Gruetter, 1979], the direct activation of guanylate cyclase by **EDRF** was also reported [Forstermann, 1986]. It was speculated that cyclic **GMP** has a causal role in the endothelium-dependent relaxation. Good correlation was repeatedly shown between the increase in cyclic **GMP** and the degree of relaxation

[Furchgott, 1984; Rapoport, 1983]. Later, little evidence was found that the relaxing agents by **EDRF** also dilate resistance vessels in a similar manner on the rings of very small arteries [Furchgott, 1987] and on perfused vascular beds [Pohl, 1987].

Many conditions and agents were found to inhibit endothelium-dependent relaxation by **Ach** in rabbit and dog arteries such as anoxia, quinacrine, eicosatetraynoic acid (**ETYA**), nordihydroguaiaretic acid (**NDGA**), p-bromophenacylbromide (**BPB**), Methylene blue and Hemoglobin [Furchgott, 1989]. Some act on the endothelial cells to interfere with the production of **EDRF**. Some were found to interact with **EDRF** itself [Griffith, 1984]. Methylene blue elicits the increase in cyclic **GMP** related to relaxation [Holzmann, 1982]. The inhibition effect of **Hb** is very much the same except that its inhibitory action can be reversed by washout from the arterial preparation [Martin, 1985; 1986].

Relaxation of an artery with endothelium-free bioassay preparation was produced by the effluents from the artery with endothelium and this relaxation can be blocked by **EDRF** inhibitors. This suggests that the basal release of **EDRF** [Griffith, 1984; Rubanyi, 1985] may be in part responsible for the development of vascular basal tone. Some **EDRF** blockers inhibit endothelium-dependent relaxation of rabbit aorta by inhibiting mitochondrial electron transport or by uncoupling oxidative phosphorylation. This interfered with the synthesis and release of **EDRF** [Griffith, 1986]. These data suggest that production of **EDRF** is dependent on mitochondrial **ATP** synthesis in endothelial cells. Therefore, the change of metabolic states, such as exercise or hypoxia, could cause the alteration of endothelium states. Studies done on perfused peripheral arteries showed that vascular smooth muscle relaxation due to intravascular hypoxia relied on the presence of endothelial cells and it was suggested that prostacyclin produced by endothelium may be involved in this dilation [Busse, 1983]. Following the observations that severe hypoxia elicited vasoconstriction in isolated coronary arteries [Borda, 1980; Rubanyi, 1985; 1986] and prostacyclin is a

vasodilator released by endothelium [Moncada, 1989], Wadsworth and co-workers [1990] attempted to investigate whether this hypoxia-induced constriction was caused by the decrease of prostacyclin in the isolated coronary artery. They found that hypoxia ( $pO_2 = 8\text{mmHg}$ ) induces a sustained contraction with the presence of endothelium and a small relaxation with the absence of endothelium. By using various receptor antagonists and enzyme inhibitors they also concluded that this reaction involved multiple mechanisms which could comprise the reduced but not abolished synthesis of prostacyclin, the release of vasoconstrictors, the release of vasodilators and **EDRF**. Moderate hypoxia produces the vasodilator from endothelium in isolated rat tail and canine femoral arteries [Busse, 1983] and in isolated cerebral arteries [Pearce, 1990]. However, severe hypoxia induces both contracting and relaxing factors from the vascular endothelium [Pearce, 1990; Wadsworth, 1990]. These conflicting results are believed to be due to the suppression of cyclooxygenase activity when  $pO_2$  values are lower than 10-15mmHg [Lands, 1978; Needleman, 1975].

In summary, **EDRF** is a very powerful and short-lived vasodilator of large and small arteries and veins. Although nitric oxide appears to be the major **EDRF**, it can not explain all endothelium-dependent responses of isolated arteries. Since intracoronary infusion of low doses of **Ach** produced coronary vasodilation and an increase in coronary sinus oxygen tension without a change in cardiac contractivity or myocardial oxygen consumption, Feigl [1990] suggested **EDRF** can be an independent vasodilator. However, the current view on **EDRF** is still confusing. The physiological role of **EDRF** is not determined yet. It is still uncertain what physiological signals trigger the release of **EDRF**. There is a lack of evidence to show that **EDRF** constitutes an initial or secondary event in metabolic vasodilation.

### 3.4.3 Potassium

A few observations have coupled  $K^+$  to the cardiac effort which links with metabolic vasoregulation. Intracoronary infusion of small doses of  $K^+$  causes

coronary vasodilation in the isolated beating heart [Bünger, 1976] and in the isolated coronary arteries [Norton, 1972; Gellai, 1974]. Probably the most convincing data defining the role of  $K^+$  was presented by Murray and co-workers [1979]. In open-chest dogs under constant – flow preparation, they compared the time courses of changes in coronary resistance and coronary venous  $K^+$  concentration following a step heart rate increase. The interstitial  $K^+$  concentration calculated by their earlier method [Murray, 1978] reached the level sufficient to account for about half of the initial vasodilation. However, the result did not support a role for  $K^+$  in maintenance of coronary vasodilation because in 6 out of 9 dogs venous  $K^+$  rose and then fell while coronary circulation was still similar in magnitude to the dilating state. The coronary vasodilatory effect of  $K^+$  was inhibited, but not abolished by ouabain [Bünger, 1976; Murray, 1978]. they suggested that  $Na^+-K^+-ATPase$  may be the  $K^+$  receptor.

In summary,  $K^+$  may play a small role in initial vasodilation due to increased heart rate. Under normal physiological conditions the importance of  $K^+$  in maintaining vascular basal tone and in both triggering and supporting metabolic vasodilation is not yet determined.

### **3.5 INTERACTION OF METABOLIC VASODILATORS**

With current technology, it is extremely difficult to set up an experimental preparation in which the independent variables do not trigger changes in the other variables which also influence vascular resistance. Thus, to understand the inter-relation and inter-reaction amongst those putative metabolic vasodilators becomes very important. Only those interactions related to my experimental work are reviewed here.

Since adenosine is the most controversial putative vasodilator and the conclusions about the role of adenosine have included to two extremes, is or not, much effort has been put into reviewing the interaction between adenosine and other vasodilators. Three questions arise:



- a) *Are they interacting, and if so, how?*
- b) *Does adenosine act as a transmitter signalling the changes of other metabolic factors? In other words, do other putative candidates regulate the coronary bed resistance through adenosine production, and the change of adenosine controls the vascular smooth muscle tone?*
- c) *Is adenosine only an intermediate metabolite? Does it affect vascular smooth muscle tone directly or only induce some changes of vascular tone which in turn finally cause an adjustment of coronary blood flow?*

### 3.5.1 CO<sub>2</sub> and O<sub>2</sub>

In Ledingham and co-workers' [1970] study, hypercapnia consistently caused a decrease of  $\dot{MVO}_2$ . It was considered as an effect separate from the vasodilator action on coronary vessels. Two reasons have been suggested for this: a) a reflexion of the general decrease in total body oxygen consumption and b) decreased myocardial contractility due to diminished pH which was caused by increased CO<sub>2</sub>. However, during increased cardiac activity the phenomenon, of pCO<sub>2</sub> causing an increase in cardiac venous pO<sub>2</sub> at any value of  $\dot{MVO}_2$ , was suggested to be due to *Bohr* shift because there was very little effect of CO<sub>2</sub> on CBF [Rooke, 1980].

### 3.5.2 CO<sub>2</sub> and Prostaglandins

To determine whether the vasodilating effect of CO<sub>2</sub> is mediated by prostaglandins, Rooke and Sparks [1980] applied indomethacin in doses sufficient to block the coronary vasodilator effect of arachidonic acid injection [Harlan, 1978]. Indomethacin did not change the relationship between pCO<sub>2</sub> and coronary oxygen delivery. Prostaglandins did not appear to mediate either the coronary vasodilation associated with increased systemic pCO<sub>2</sub>.

### 3.5.3 Adenosine and Oxygen

Berne [1963; 1964] has shown that anoxia and severe hypoxia result in the appearance of inosine and hypoxanthine in the cardiac perfusate. It was suggested that adenosine acts as a transmitter signalling the oxygen tension at a critical site in the myocardial cell to adjust the vascular muscle tone. When employing an inhibitor of adenosine deaminase, 8-azaguanine, adenosine was detected in the perfusate of isolated cat and guinea pig hearts in quantities proportional to the increase in coronary flow due to graded hypoxia [Berne, 1964; Katori, 1966]. Stronger evidence was provided by Schrader *et al* [1977] that both perfusate adenosine and  $^{14}\text{C}$ -adenosine are quantitatively increased subject to different degrees of hypoxia. However coronary vasodilation induced by hypoxia was not altered by intravenous aminophylline injection. In these experiments the coronary artery was not maximally dilated, tested by exogenous adenosine infusion during hypoxia. The result does not support the hypothesis that adenosine is responsible for vasodilation during hypoxia [Afonso, 1972]. To study the interaction between the effects of oxygen and adenosine on helical coronary arterial strips contractile tension induced by acetylcholine, Gellai [1973] observed that adenosine-induced relaxation is augmented in inverse proportion to tissue bath  $\text{pO}_2$ , and in the absence of adenosine in the tissue bath the change of  $\text{pO}_2$  has no effect on the contractile tension of the strip. This result led them to propose that  $\text{pO}_2$  plays a direct role in regulating coronary vascular tone with the presence of adenosine which may have a constant level in vivo.

### 3.5.4 Adenosine and $\text{CO}_2$ and pH

Based on the findings that the intracoronary infusion of adenosine on dogs led to an increase in glucose uptake, to the appearance of myocardial lipolysis and to a release of  $\text{H}^+$  ions and total  $\text{CO}_2$  from the heart, Raberger [1970] proposed that adenosine leads to a metabolic acidosis and via changes in the myocardial  $\text{pCO}_2$  to coronary vasodilation. To test this hypothesis, the effects of an intracoronary

application of adenosine on **CBF** were investigated over a wide range of arterial pH and  $p\text{CO}_2$  and at different degrees of extra- and intra-cellular buffer capacities [Raberger, 1971]. The results show a significant dependence of the coronary vasodilatory action of adenosine on the arterial pH when concomitant changes in extracellular buffer capacity and changes in arterial  $p\text{CO}_2$  lead to a reciprocal change in intracellular pH and coronary vascular conductance. However this does not exclude the possibility that both adenosine and pH or  $\text{CO}_2$  have a separate effect on vascular smooth muscle.

#### **3.5.5 Adenosine and Prostaglandins**

Since Minkes *et al* [1973] observed that the adenosine nucleotides **ATP** and **ADP** are potent releasers of a prostaglandin-like material from the rabbit heart, prostaglandins, the adenine nucleotides and their derivatives may interact in the regulation of **CBF** [Alexander, 1975] as far as the vasoactive effect of adenosine and prostaglandins is concerned. However, the use of indomethacin failed to alter the exogenous adenosine dose-response curve in heart-lung preparation [Alexander, 1975].

## **CHAPTER 4**

### **METHODS**

## 4.1 MATERIALS AND INSTRUMENTS

### 4.1.1 Experimental Animals

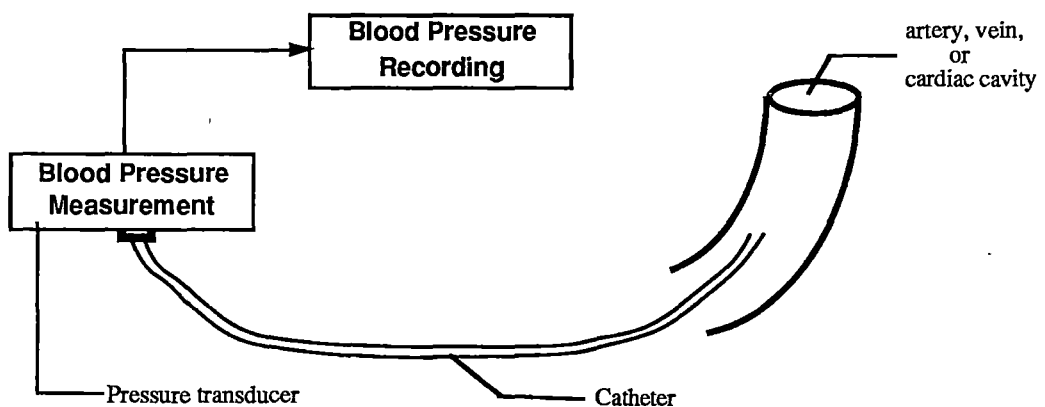
Sheep bred on the University of Tasmania's experimental farm with the following general characteristics were used:

Species:	Polworth(ram)-Comeback (ewe) cross
Sex:	both
Weight :	23-35Kg
Age:	6-9 months

### 4.1.2 Experimental Instruments and Their Calibrations

#### 4.1.2.1 Blood Pressure Measurements

Statham P 23ID pressure transducers (Statham Laboratory Inc.) were used for blood pressure measurements with size 7 catheters. The system used to obtain measurements is shown in Figure 4.1:



**Fig.4.1:** Diagrammatic representation for blood pressure measurements

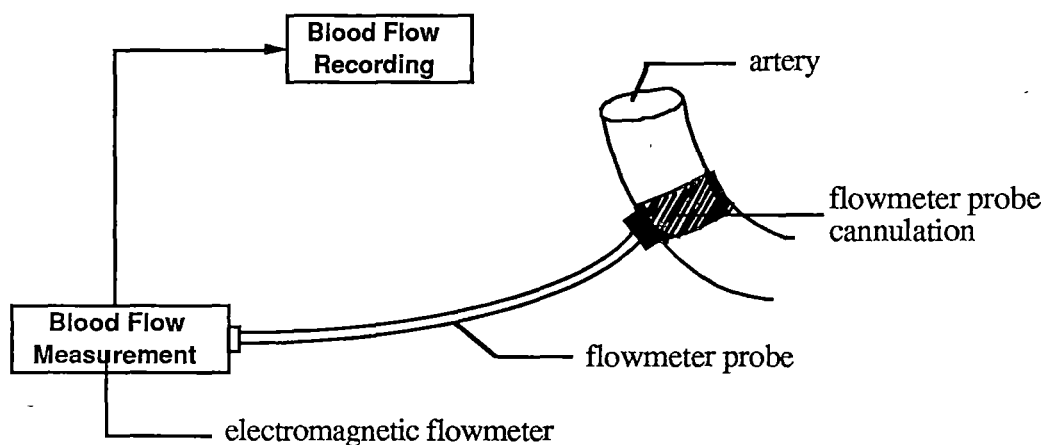
The pressure sensing element in the transducers is a semi-conductor strain-gauge, which linearly converts pressure to voltage over a restricted range. A check that all the transducers used had a linear pressure-voltage relationship in the pressure

range 0 - 180mmHg is included in Appendix A. Only those transducers showing a linear calibration ( $R \geq 0.99$ ) in the pressure range 0-180mmHg were used in this work.

7F catheters with side holes were used. The level of transducer was kept to the tip of the catheter as close to the centre of the right atrium as possible. However, in practice, it is not possible to get an absolute zero value. As far as my research is concerned, the difference of data is more important than the absolute value among pressure, flow and oxygen saturation. Thus, a small shift of zero baseline will not affect the analysis of the data as long as the instrument is correctly calibrated. It is essential when using these transducers to keep the cardiac catheter free of thromboses. High doses of heparin (initial dose 500 $\mu$ /Kg, I.A., then about 250  $\mu$ /Kg, I.A. every 1-2 hours) were used to ensure there was no clotting during experiments.

#### 4.1.2.2 Blood Flow Measurements

Various sizes of NARCO electromagnetic flowmeters were used for coronary blood flow and pulmonary artery flow measurements. The system is illustrated in Figure 4.2:



**Fig.4.2:** Diagram for blood flow measurements

The principle for electromagnetic flowmeters is based on the *Faraday* effect [Woodcock, 1975]: when a magnetic field is applied at right angles to the direction of motion of a conducting field, a potential difference is set up at right angles to both the

flow direction and the magnetic field. To reduce experimental artifacts such as that due to polarization of the sensing electrodes, and to improve the signal-noise ratio, the **NARCO** flowmeters use an alternating magnetic field. The flowmeters used have linear calibration curves passing through zero and equal sensitivities with forward and backward flow.

I relied on the calibration supplied by the manufacturer with each probe. The internal calibration system of the flowmeter was used to calibrate the data acquisition system. Before each experiment, the flowmeter probes were cleaned thoroughly and soaked in saline for at least 20 mins. The flowmeter was calibrated according to the manufacturer's instructions and the offset carefully minimized according to calibration factors supplied for each probe before cannulating the probes on vessels. After cannulation, the vessel was repeatedly occluded to determine the zero flow. The surroundings of the flowmeter probe are kept as dry as possible to avoid pick up of spurious signals by the system. After finishing an experiment, the calibration was carefully performed again to assure the validity of the previous calibration.

#### **4.1.2.3 Metabolic States Measurements**

A fibre optic technique is used to continuously measure coronary sinus O<sub>2</sub> saturation (SO<sub>2</sub>). An **OXIMETRIX** Oximetry System (Abbott Laboratories, U.S.A.) was loaned by the Intensive Care Unit of Royal Hobart Hospital. The system contains three components: a fibre-optic catheter, optical module and computer. The optical module receives reflected light signals, converts them to electrical signals and amplifies them for transmission to the computer which computes SO<sub>2</sub> values.

An **ABL3** Acid-Base Laboratory was used for pH and blood gas analysis. Blood samples were drawn into iced heparinized plastic syringes. Blood was kept in

ice immediately after withdrawing. Measurements were carried out on the **ABL3** previously calibrated with known gas mixtures.

#### **4.1.2.4 Data Recording**

Analog signals from the pressure transducers, flow meters and oxygen analyzer were amplified with an 8-channel polygraph (**Grass Co.**) and plotted on conventional chart paper during experiments.

In the early experiments, a 7-channel analog tape recorder (**Racal Recorders Ltd, Store 7 DS**) was used to record all the data, which was later digitized for computer analysis. The relationship of input to output in the tape recorder was carefully checked for all 7 channels to ensure that it was linear to within the resolution of the analogue-to-digital converter (about 1 part in 500) (See appendix B).

In later experiments, signals from the polygraph amplifier were directly digitized during experiments with an analogue-to-digital interface (**NB-DMA-8, NI-488** for Macintosh - series number 3643 and **LabView** software: National Instruments) in a Macintosh II computer (Apple Corporation).

#### **4.1.2.5 Computer Analysis**

Data from the analogue tape recorder were digitized with an 8-channel 10-bit analogue-to-digital converter (Central Science Laboratories, University of Tasmania) and transferred to a **PDP11** computer (Digital Equipment Corporation) running the Unix Operating System via a **DMA** board. Calibration factors were derived from digitized data obtained while calibrating the transducers. These factors were applied to the raw digitized data before further processing.

In later experiments, data digitized directly on the **Macintosh II** using the **LabView** software were transferred to a **SUN** workstation where they were decoded, calibrations were applied and then processed.



The polygraph amplifiers are configured as low frequency pass amplifiers with an adjustable high frequency cut-off point usually set at 30Hz. When digitizing, data were sampled at 10ms intervals, providing approximately 3x over-sampling.

A variety of programs were written on the **PDP11** and **Sun** Unix systems to process the data, fit parameters and display results as follow:

- |                       |  |
|-----------------------|--|
| <b><i>decal</i></b>   | decodes the binary data collected by the <b>LabView</b> system and calibrates it on the <b>Sun</b> Unix systems.   |
| <b><i>mindst</i></b>  | Uses left ventricle pressure to separate each beat into systolic and diastolic phases, the mean diastolic and systolic data (including flow, pressure, coronary resistance and cardiac work) is then calculated on a beat by beat basis. |
| <b><i>xu4</i></b>     | finds the offset point of stimulus of aortic constriction, where proximal aortic pressure has come back to control value, for further decay calibration.   |
| <b><i>expfit</i></b>  | finds the highest value of mean coronary blood flow or lowest value of coronary resistance due to hypoxia for further analysis <sup>1</sup> .  |
| <b><i>congrad</i></b> | uses Powell's conjugate gradient minimisation technique [Press, 1988] to determine the best values of a, b, k to fit a curve " $y = b + a e^{-kt}$ ".  |

#### 4.1.2.6 Statistical Analyses

Some statistical calculations were carried out on the Macintosh:

- |                     |  |
|---------------------|--|
| <b><i>Excel</i></b> | was used to store blood gas analysis data and half lives and calculate the mean, standard deviation ( <b>SD</b> ), standard error of mean ( <b>SEM</b> ), and slope in linear regression and other calculations. |
|---------------------|--|

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<sup>1</sup>*expfit* was originally used for quickly determining approximate decay times by linearizing data, but the *congrad* programme provided better results with noisy data.

**StatView**

was used to do two-tailed pair student's *t* tests and calculate the mean and SEM.

**Criquet Graph**

was used to graph the data for presentation and compare two groups of data by using linear regression.

### 4.1.3 Drugs and Chemicals

**Table 4.1:** Drugs and chemicals for this investigation

Name	Supplier	Use
Pentobarbitone	Sanofi	induction and continuous anaesthesia
Sodium Bicarbonate	R.H.H. Pharmacy	intravenous infusion when needed to correct metabolic acidosis due to anaesthesia
Physiological Saline	R.H.H. Pharmacy	1) slow drip into jugular vein to compensate the loss of body liquid during experiments
		2) flushing all the lines to prevent coagulation of blood in catheters
		3) some drug or chemical solutions
		4) soak flowmeter probes for calibration
Heparin	R.H.H. Pharmacy	systemically for sheep to avoid blood coagulation
Ethanol and Sodium Hydroxide	Sigma	dissolving 8-phenyltheophylline
Phentolamine	R.H.H. Pharmacy	blocking adrenergic $\alpha$ -receptor (1mg/Kg, I.V.)
Propranolol	Sigma	blocking $\beta$ -receptor (2mg/Kg, I.V.)
8-phenyltheophylline	Sigma	adenosine competitive antagonist (7.5mg/Kg, I.V.)
adenosine	Sigma;	check the blocking effect of 8-phenyltheophylline
nonadrenaline	Sigma;	check the blocking effect of phentolamine
adrenalin	Sigma;	check the blocking effect of $\alpha$ , $\beta$ blockers
isoprenaline	Sigma;	check the blocking effect of propranolol

## 4.2 EXPERIMENTAL METHODS

### 4.2.1 Animal Preparation and Experimental Setup

Sheep were studied in an acute, open-chest preparation. They were anaesthetized with pentobarbitone (35mg/kg I.V. bolus). Anaesthesia was maintained with continuous intravenous pentobarbitone injection (4mg/kg/hour) through a jugular vein catheter. The animals were artificially ventilated (3.5L/min) by a positive

pressure respirator via a cuffed tracheal tube and a non-rebreathing system. Inspired air was supplemented with oxygen so that arterial oxygen tension remained between 100 and 150mmHg. Tidal volume and respiratory rate were constant during each experiment. Carbon dioxide tension was kept at  $30\pm 5$ mmHg. Arterial pH was maintained at  $7.45\pm 0.05$  by a supplement of sodium bicarbonate through intravenous infusion when needed, although the efficiency of this manoeuvre was not determined.

The heart was exposed through a left lateral thoracotomy by removing the fourth left rib. The pericardium was incised and the heart was suspended in a pericardial cradle. The pulmonary artery and coronary circumflex artery were carefully separated for electromagnetic flowmeter probe cannulation. The proximal aorta was dissected for aortic band cannulation which will be fully described in the next section). Sheep were instrumented as indicated in Table 4.2.

**Table 4.2:** Experimental instruments and their purpose (see text for detail)

<b>PRESSURE</b>	<b>INTRUMENTS</b>
Aorta	#7 fluid filled catheter
Left Ventricle	#7 fluid filled catheter
Coronary Sinus	#7 fluid filled catheter
Carotid Artery	Arterial introducer (for aortic catheter)
Venous	any catheter as delivery lines
<b>FLOW</b>	
Pulmonary	16 NARCO RT 500
Coronary circumflex	2 - 3.5mm NARCO RT 500
<b>OXIMETER</b>	
Coronary Sinus	#7 fluid filled fibre optic catheter
<b>CARDIAC O<sub>2</sub> USAGE</b>	
Proximal Aorta	Same catheter as for PRESSURE
Coronary Sinus	Same catheter as for SO <sub>2</sub>

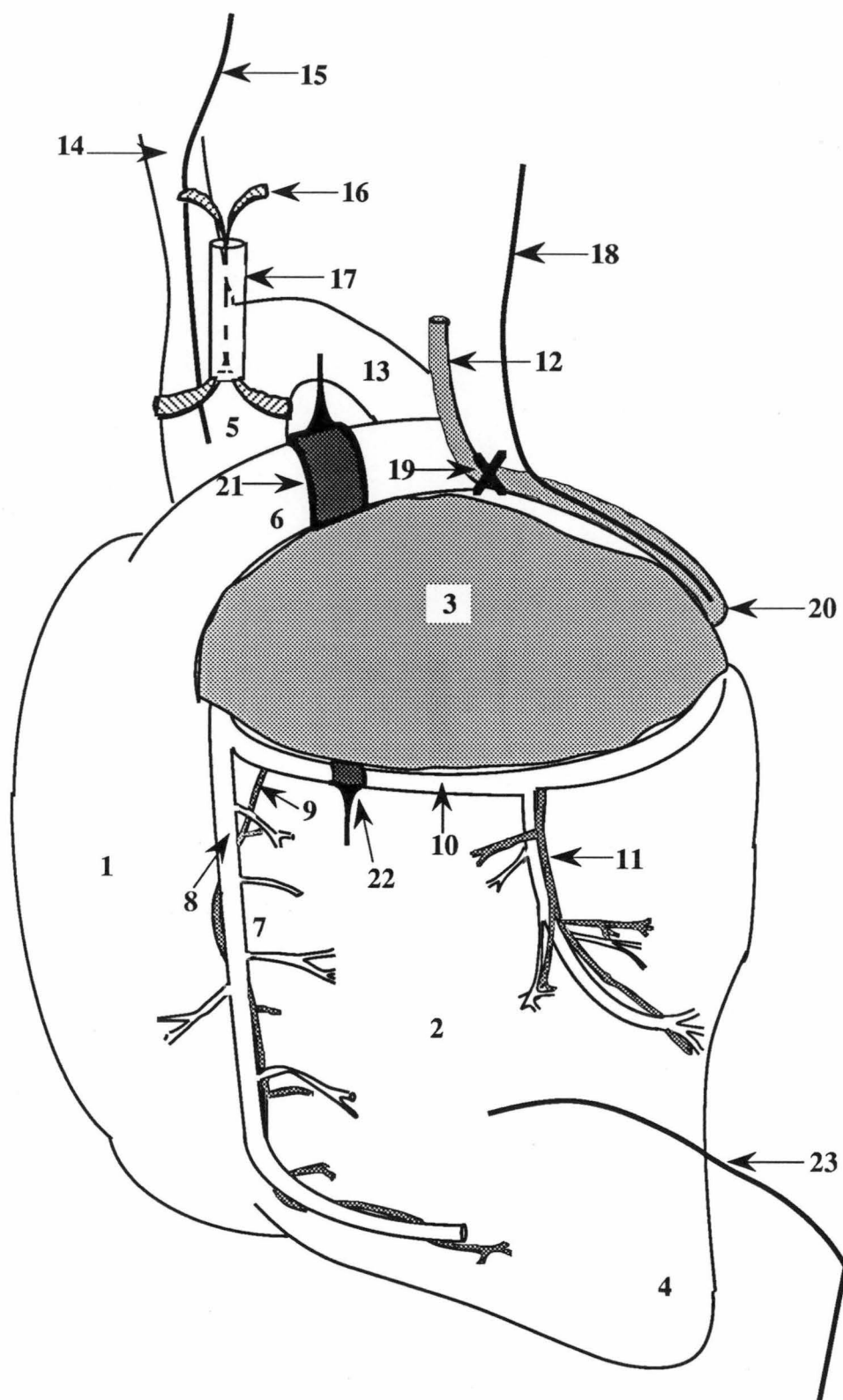
Proximal aortic pressure was measured with a Statham P 23ID transducer via a 7F catheter side hole through an introducer in the left carotid artery. Left ventricular pressure was measured with a pressure transducer via a 7F catheter through the left ventricular wall. The hemiazygous vein was isolated (the anatomy of sheep is included at appendix C). A 7F catheter (or a fibre-optic catheter) was inserted into the coronary sinus through the hemiazygous vein which was then occluded beyond the insertion site, so that coronary sinus pressure could be recorded by using a pressure transducer and coronary sinus blood could be taken at the same time as the proximal aortic sample for hemoglobin and blood gas analysis. 16mm and 2-3.5mm electromagnetic flowmeter probes were placed separately on the pulmonary artery and the coronary circumflex artery. Coronary sinus O<sub>2</sub> saturation was also continuously detected by a fibre optic catheter. The instrumented sheep heart is shown in Figure 4.3. All animals were anticoagulated with heparin sodium and kept warm by using an electrical heater during experiments. After completion of the studies, the sheep were killed with a lethal dose of sodium pentobarbital. The position of the coronary sinus cannula tip was determined by postmortem and ranged from 3–4cm from the coronary sinus ostium.

#### **4.2.2 Stimulus**

Pressure rise and hypoxia were the interventions chosen for this study.

##### ***Aortic band cannulation***

A length of fibreglass measuring tape and a plastic tube were used to make an aortic band which was cannulated on the proximal aorta as shown in Figure 4.4. This measurable band enabled the aortic cross-sectional area to be decreased by a measurable amount for each experimental run.

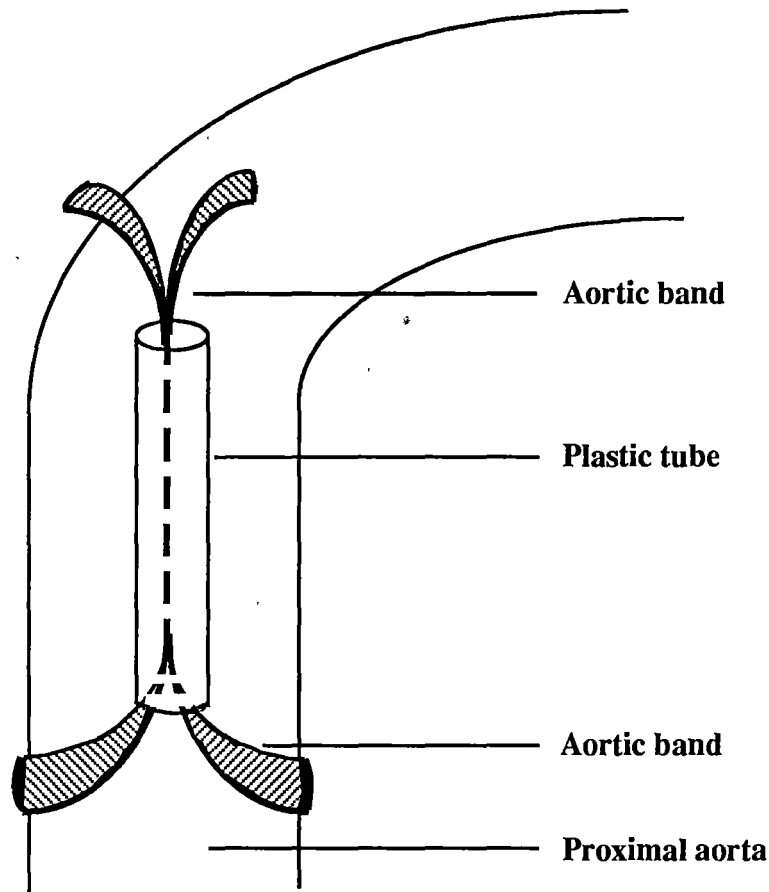


**Fig 4.3:** Diagrammatic representation of the instrumented sheep heart (1. Right Ventricle; 2. Left Ventricle; 3. Left Atrium; 4. Apex; 5 Aorta; 6. Pulmonary Artery; 7. Longitudinal Groove; 8. Left Anterior Descendants; 9. Great Vein; 10. Circumflex Artery; 11. Intermediate Groove; 12. Hemiazygos Vein; 13. Descending Aorta; 14. Left Carotid Artery; 15. Catheter; 16. Aortic Band; 17. Plastic Tube; 18. Catheter; 19. Occluder to block the venous blood from the the lung; 20. Coronary Sinus; 21. Pulmonary Flowmeter Probe; 22. Circumflex Flowmeter Probe; 23. Cathether in LV).

### ***Low oxygen percentage mixed gas***

Three types of mixed gas were used to systemically decrease oxygen supply:

- Type I:** pure nitrogen
- Type II:** 5% of oxygen + 95% of nitrogen
- Type III:** 5% of oxygen + 5% of carbon dioxide + 90% of nitrogen



**Fig.4.4:** Diagrammatic representation of the aortic band. (see text for explanation)

#### **4.2.3 Outline of Research Protocol**

After a sheep was set up as described in 4.2.1 and control data were obtained, the interventions were applied. Hemodynamic variables were continuously monitored before each intervention to ensure that steady-state resting conditions had been achieved. When the animals had achieved steady-state hemodynamic conditions, both aortic and coronary sinus (CS) blood samples (1.0ml each) were taken anaerobically

for measurement of hemoglobin, pH, pCO<sub>2</sub>, pO<sub>2</sub>, SO<sub>2</sub>, and Na<sup>+</sup> and K<sup>+</sup>. Samples were also withdrawn at intervals through experiments for blood gas and electrolytic balance measurements. Blood flow, pressure and CSSO<sub>2</sub> were measured continuously through the control period, the period of intervention and the recovery period as shown in Figure 4.5. At least 20 heart beats of control data were collected and the whole period of recovery from offset to steady-state was taken.

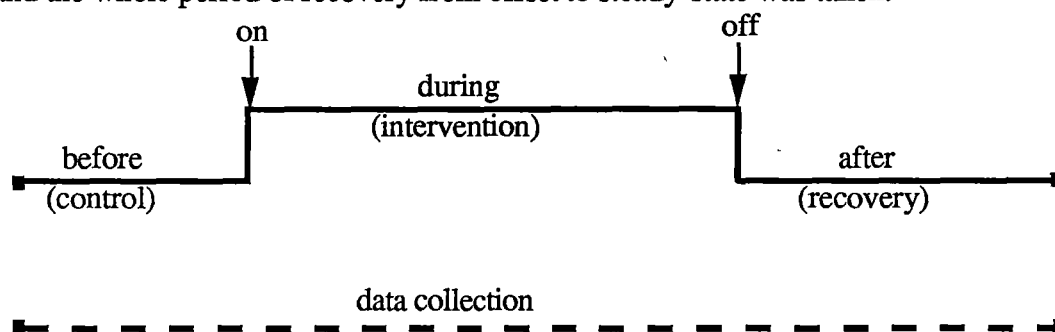


Fig.4.5: Diagram for illustration of the period of data collection

Data were recorded both on chart paper by the polygraph for quick checking during experiments and by analog tape recorder for further analysis on computer. Some experiments were digitized on a Mac II by LabView system in the operating theatre. There were at least three runs for the same intervention. For instance, I recorded at least three runs increasing proximal aortic pressure by 20% lasting 2mins. Runs at different pressure increases and different durations were performed in a random order.

Using paper chart-recording data as reference, data were digitized<sup>1</sup> from the analog tape recorder using the data acquisition programme *decal* and stored on the computer. Using the computer, continuous coronary resistance, cardiac work and the half life of decay of response due to intervention were calculated.

<sup>1</sup>The data were digitized at 10ms sampling intervals in all cases.

The same protocol was carried out for each sheep before and after adenosine,  $\alpha$  and  $\beta$  receptor inhibitions. Each group of experiments used at least 6 sheep.

### **4.3 DATA ANALYSIS**

#### **4.3.1 Data Rejected (Experimental Criteria)**

a) During the experiment, a 2-3second coronary artery occlusion was performed to ensure that the coronary circumflex flowmeter baseline had remained stable. Data were rejected if zero flow measurement was shifted more than 5% of the normal flow value. Otherwise, if the flowmeter showed a constant zero-flow offset, it would be extracted during analysis.

b) Noisy data were rejected.

c) Data were rejected if During steady-state conditions the pH lay outside the range 7.35 – 7.45 to ensure all the experimental runs started from a common baseline, except the experiments discussed in chapter 6.

d) If the aortic pressure in the resting period changed more than 20% except when using  $\alpha$  and  $\beta$  blockers, data were rejected.

e) If any of the hemodynamic variables failed to achieved a new steady-state baseline, data were discarded.

#### **4.3.2 Calculation of Derived Parameters**

##### **4.3.2.1 Calculation of Coronary Resistance, Cardiac Work and $\dot{MVO}_2$**

After experimental data had been collected, digitized, calibrated and stored in the computer, coronary resistance (cr) and cardiac work (cw) for each sample were calculated using equations 4.1 and 4.2.



$$CR = \frac{Paop - \frac{LVP + CSP}{2}}{CQ} \quad (4.1)$$

where:

**CR** – coronary resistance;  
**Paop** – proximal aortic pressure;  
**LVP** – left ventricular pressure;  
**CSP** – coronary sinus pressure; and  
**CQ** – coronary flow.

$$\dot{w}_p = PF \quad (4.2)$$

where:

$\dot{w}_p$  – the rate of potential work;  
**P** – pressure of proximal aorta; and  
**F** – flow rate of cardiac output (mean pulmonary artery flow).

Myocardial oxygen consumption ( $\dot{MVO}_2$ ) was calculated by equation 4.3 using the data both from computer and blood gases measurements.

$$\dot{MVO}_2 = CBF \times (A - V) O_2 \text{ content} \quad (4.3)$$

where:

**CBF** – coronary blood flow;  
**A** – artery;  
**V** – venous;  
**(A-V) O<sub>2</sub> content** – arteriovenous oxygen content difference.

#### 4.3.2.2 Calculation of Half Life

A representation of a typical experimental trace is shown in Figure 4.6.

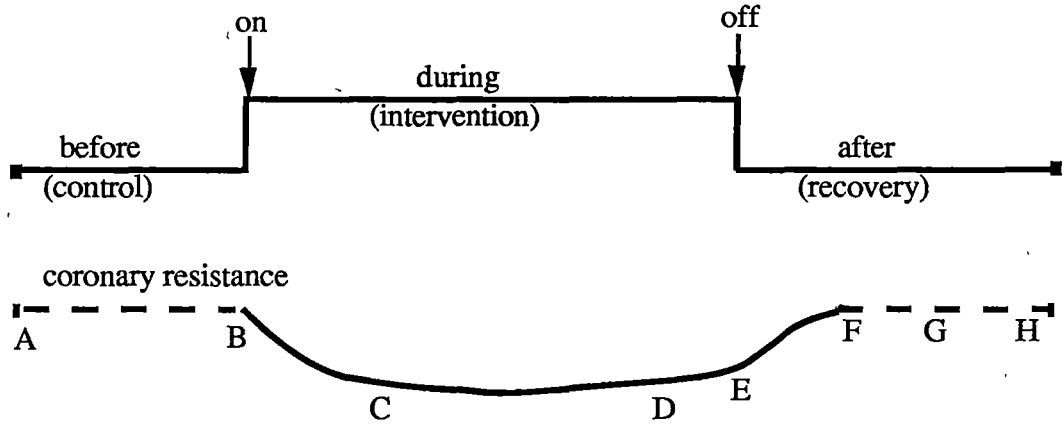


Fig.4.6: Diagram for illustration of half life calculation

where A-B is the control baseline, B is the intervention starting point, C is the point where *cr* stabilizes after intervention, C-D is the stable period of *cr* during intervention, E is the point intervention ceases, E-F is the period of recovery of the *cr*, G is the point *cr* returns to a stable value, G-H is a stable *cr*. If we can assume first-order decay kinetics, then we expect that the curve E-F-G-H will have the equation:

$$R(t) = R_b + R_0 e^{-kt} \quad (4.4)$$

where  $R(t)$  is the effect of the response at time  $t$  after point E,  $R_0$  is a constant which represents the response caused by the intervention in the period C-D,  $R_b$  is the baseline response (represented by the section G-H),  $k$  ( $s^{-1}$ ) is the rate constant for the decay of the response.

The half-life of the response,  $t_{1/2}$ , is defined as the time for the response to decay to half its original value (relative to the baseline value  $R_b$ ), *ie* at  $t_{1/2}$

$$R(t_{1/2}) = \frac{1}{2} R_0 + R_b.$$

Values of  $k$  were found by fitting<sup>1</sup> Equation 4.4 to the curve marked E-F-G-H<sup>2</sup>.  
Then half life:

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \quad (4.5)$$

### 4.3.3 Analysis Protocol

After  $cr$  and  $cw$  were calculated and exponential decay curves fitted to the decay portions of the data to derive  $t_{\frac{1}{2}}$  values, the following analyses were performed:

- a) *Calculated  $t_{\frac{1}{2}}$  values were compared with different degrees and durations of aortic constrictions. The degree of aortic stenosis was measured by the change of proximal systolic pressure (refer to chapter 5).*
- b) *Correlations between  $cr$  and metabolic parameters during systemic hypoxia were studied (refer to chapter 6).*
- c) *The mean diastolic coronary flow and coronary resistance were separately compared with the coronary sinus oxygen saturation in either increased cardiac work or decreased systemic oxygen supply (refer to chapter 7).*
- d) *The  $t_{\frac{1}{2}}$  of the vasodilating effect of intracoronary adenosine infusion was determined at several dose levels with different sheep (refer to chapter 8).*
- e) *The procedure described above was repeated after using adenosine,  $\alpha$  and  $\beta$  blockers (refer to chapters 6, 7, 8, 9).*

### 4.3.4 Statistical Analysis

The instrument and computer calibrations have been fully explained in section 4.1.2.

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<sup>1</sup>Due to the noisy experimental data, I used Powell's conjugate gradient minimisation technique to fit all three parameters  $R_b$ ,  $R_a$ ,  $k$  simultaneously. A least-squares criterion—minimum ( $\sum (X_i^{\text{observed}} - X_i^{\text{calculated}})^2$ ) was used to select values  $R_b$ ,  $R_a$  and  $k$  for best fit.

<sup>2</sup>Examples of the data fitting technique are found in a later section - actual protocols for different aims

Mean, standard deviations and standard errors were applied for data analysis. The standard errors of mean (SEM) presented in the figures and tables represent the variability between animals [degree of freedom (d.f.) =  $n - 1$ , for  $n$  animals].

In some experiments, it was necessary to determine whether two slopes were significantly different. The two slopes ( $S_1, S_2$ ) from separate linear regression fits were compared for parallelism [Kleinbaum, 1988]; three alternative hypotheses can be found:

$$S_1 > S_2; \quad S_1 < S_2; \quad S_1 \neq S_2$$

The test statistic for evaluating parallelism is then given by:

$$T = \frac{\hat{S}_1 - \hat{S}_2}{SD_{(\hat{S}_1 - \hat{S}_2)}} \quad (4.6)$$

where:  $\hat{S}_1$  = least-squares estimate of the slope  $S_1$  using the  $n_1$  observations  
 $\hat{S}_2$  = least-squares estimate of the slope  $S_2$  using the  $n_2$  observations  
 $SD_{(\hat{S}_1 - \hat{S}_2)}$  = estimate of the standard deviation of the estimated difference between slopes  $(\hat{S}_1 - \hat{S}_2)$

where  $SD_{(\hat{S}_1 - \hat{S}_2)}$  is equal to the square root of the following variance:

$$SD_{\hat{S}_1 - \hat{S}_2}^2 = S_{P,Y/X}^2 \left[ \frac{1}{(n_1 - 1)S_{X1}^2} + \frac{1}{(n_2 - 1)S_{X2}^2} \right] \quad (4.7)$$

where

$$S_{P,Y/X}^2 = \frac{(n_1 - 2)S_{Y/X1}^2 + (n_2 - 2)S_{Y/X2}^2}{n_1 + n_2 - 4} \quad (4.8)$$

where

$S_{Y/X1}^2$  = residual mean-square error for the group1 data

$S_{Y/X2}^2$  = residual mean-square error for the group2 data

$S_{X1}^2$  = variance of the X's for the group1 data

$S_{X2}^2$  = variance of the X's for the group2 data

The test statistic given by 4.6 will be distributed as a Student's  $t$  with  $n_1 + n_2 - 4$  degrees of freedom when the hypotheses is true. The following critical regions are for different hypotheses and significance level  $\alpha$ :

$$T \geq t_{n_1+n_2-4, 1-\alpha} \quad \text{for } S_1 > S_2$$

$$T \leq t_{n_1+n_2-4, 1-\alpha} \quad \text{for } S_1 < S_2$$

$$|T| \geq t_{n_1+n_2-4, 1-\alpha/2} \quad \text{for } S_1 \neq S_2$$

#### 4.4 ACTUAL PROTOCOLS

##### 4.4.1 Actual Protocol for Chapter Five

###### 4.4.1.1 Aims

The aim of these experiments was to investigate whether common mechanisms exist in coronary vasoregulation due to exercise. The time course of decay ( $t_{1/2}$ ) of both coronary resistance and diastolic coronary blood flow was determined in response to the different degrees and durations of left ventricular afterload before and after  $\alpha$ ,  $\beta$  and adenosine receptor inhibitions.

###### 4.4.1.2 Method

The sheep experimental setup was fully described in section 4.2. 13 (out of 17) successful animal experiments were used as a control group in which no inhibitors were added. Similar measurements were obtained from 7 (out of 10) sheep before and after adenosine,  $\alpha$  and  $\beta$  receptor inhibitions. Other animals died unexpectedly or had various problems with instruments during experiments. The experiments are summarized in Table 4.3.

Table 4.3: Number of sheep for chapter 5 experimental work

	<b>control (group1)</b>	<b>inhibition (group2)</b>		
		<b>control</b>	<b>adenosine inhibition</b>	<b>adenosine+<math>\alpha</math>-, <math>\beta</math>-adrenergic inhibitions</b>
No. of sheep	17	10	10	10
successful experiments	13	7	7	5

### *Group 1*

This is a pure control group. After the sheep had been set up and all recording instruments connected, it was allowed to rest for 30 minutes. Hemodynamic variables were continuously monitored and arterial blood-gas analysis was performed during this period to ensure that steady-state resting conditions had been achieved. After obtaining resting hemodynamic measurements, proximal aortic systolic pressure was increased by approximately 15%, 30% and 45% for the different durations: 30-, 60-, 90- and 120-seconds. The interventions were applied in random order and each intervention was repeated at least 3 times for every sheep. An interval of at least 10minutes was allowed between runs, so that after each run, the sheep were given sufficient time to rest until the hemodynamic variables recovered to stable values<sup>1</sup>.

### *Group 2*

This group consisted of 7 successful experiments for both control and adenosine inhibition, 5 successful experiments for fully blocked study including both adenosine,  $\alpha$ - and  $\beta$ -receptor inhibitions. Sheep were instrumented with

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<sup>1</sup>It is almost impossible for all the hemodynamic variables to recover completely to the original steady-state resting conditions in my preparation, thus, new steady-state resting conditions were required.

intracoronary cannulation<sup>1</sup> for intracoronary adenosine injection in additions to the basic setup described in section 4.2. When the animals had achieved steady-state hemodynamic and arterial base-acid balance conditions, a similar protocol to that used for group1, but the intervention times were all 120seconds. Thereafter, the dose-response curve of intracoronary bolus administration of adenosine<sup>2</sup> was determined before and after intravenous administration of 8-phenyltheophylline (8-PT: 7.5mg/kg)<sup>3</sup> to ensure the proper blocking effect had been achieved. 8-PT was injected slowly due to the high concentration of ethanol and sodium hydroxide in the solution. Generally 45minutes<sup>4</sup> were required for 8-PT to produce more than 80% blocking effect in sheep. Immediately thereafter, the aortic pressure increase protocol was repeated in random order. Finally, adenosine dose-response curves were observed. Following the intravenous injections of phentolamine (1mg/kg)<sup>5</sup> and propranolol (2mg/kg)<sup>6</sup> as  $\alpha$ - and  $\beta$ -adrenergic receptor blockers, the aortic pressure increase protocol was repeated again. Due to the vasoregulatory effect of both  $\alpha$ - and  $\beta$ -blockers, the hemodynamic variables showed a new baseline which demonstrated low heart rate, low pressure and low cardiac contractility. However, as long as a steady-state had been achieved, the above protocol was repeated.

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<sup>1</sup>This will be fully described in section 4.4.4.

<sup>2</sup>Refer to later section 4.4.4.

<sup>3</sup>8-PT was dissolved in the solution (3 part absolute ethanol + 1 part 1M NaOH by volume) to make a concentration of 50mg/ml for intravenous administration.

<sup>4</sup>Maximal blocking effect of 8-PT was invariably observed within 45mins.

<sup>5</sup> The original commercial saturation (10mg/ml) was used.

<sup>6</sup> Diluted with isotonic saline to a concentration of 5mg/ml for intravenous administration.

#### 4.4.1.3 Data Analysis

Following the experimental criteria set in section 4.3.1 and after ensuring there were no distortions of computerised data, data were selected for further analysis. Three degrees of proximal aortic constriction were carried out for the varying durations. Figure 4.7 demonstrated the proximal aortic pressure changes caused by the different degrees of constriction. Equations 4.1 and 4.2 were used to estimate **cr** and **cw** (the only contributor to the change of **cw** was the change of proximal aortic systolic pressure because the cardiac output, mean pulmonary artery blood flow, was not changed). Figure 4.8(Aa,b,c) show the measured and computed hemodynamic variables in response to the aortic constriction stimulus. The calculated mean diastolic and systolic data<sup>1</sup> were plotted against time beat by beat on Figure 4.8(B) from the data of Figure 4.8(A). The onset and offset of **CBF** and **cr** due to the interventions can be seen. The following abbreviations are used:

<b>cqd</b>	mean diastolic coronary blood flow
<b>cqm</b>	mean coronary blood flow
<b>cr</b>	mean diastolic coronary resistance
<b>s.aop</b>	systolic proximal aortic pressure
<b>cw</b>	mean calculated cardiac work
<b>LVDP</b>	left ventricular end diastolic pressure
<b>d.aop</b>	diastolic proximal aortic pressure
<b>pqm</b>	pulmonary artery mean flow

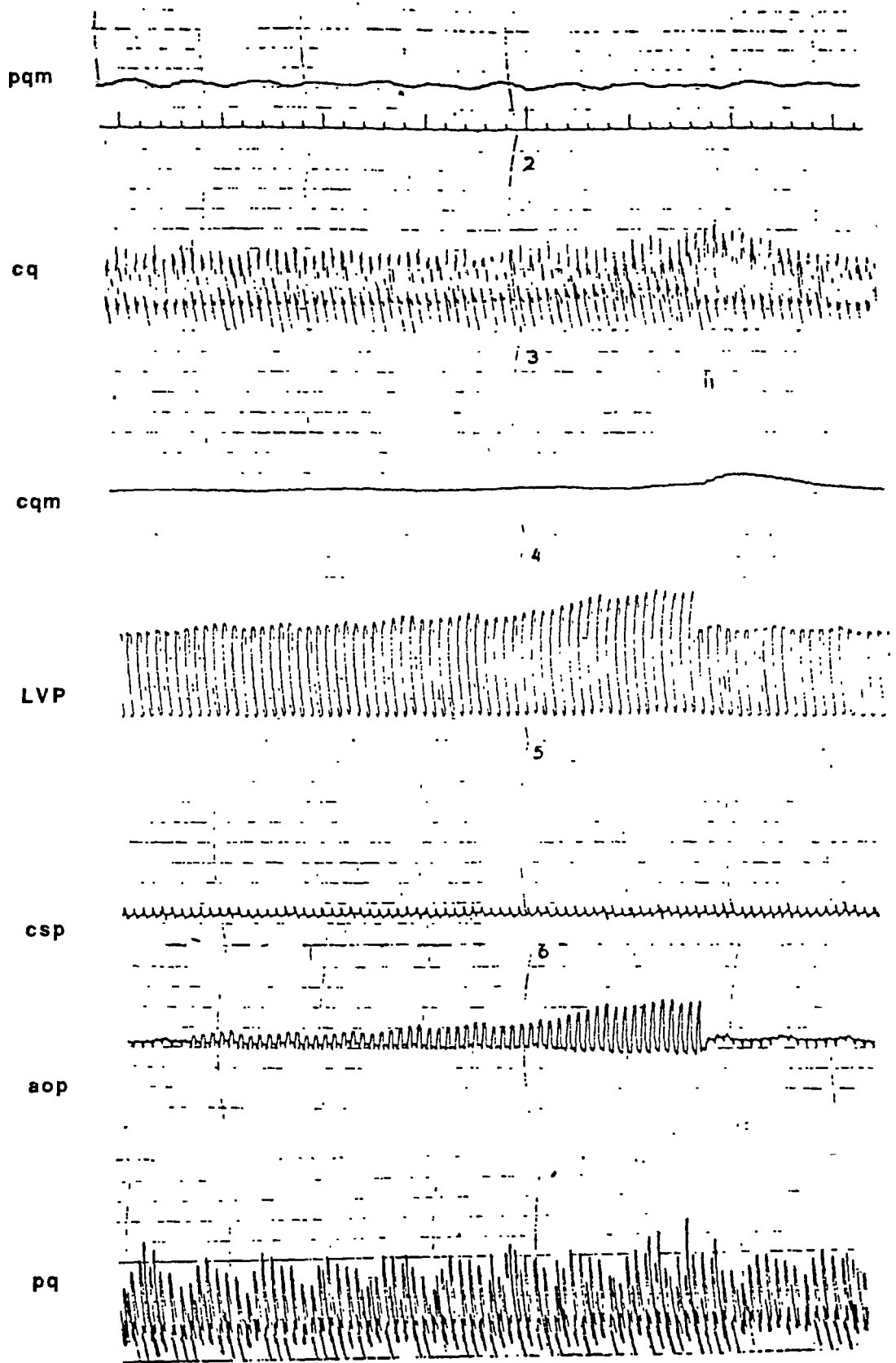
In this case, the aortic constriction induced a 43% increase in aortic pressure and lasted 120 seconds. Data were collected for 210 seconds, which included the data before intervention, during, and after intervention<sup>2</sup>. Figure 4.8 also demonstrates there are no obvious changes in cardiac output (**pqm**), **LVDP** and **d.aop** during intervention.

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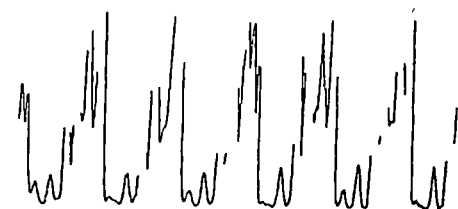
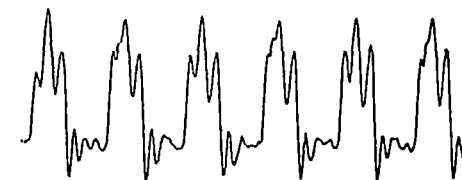
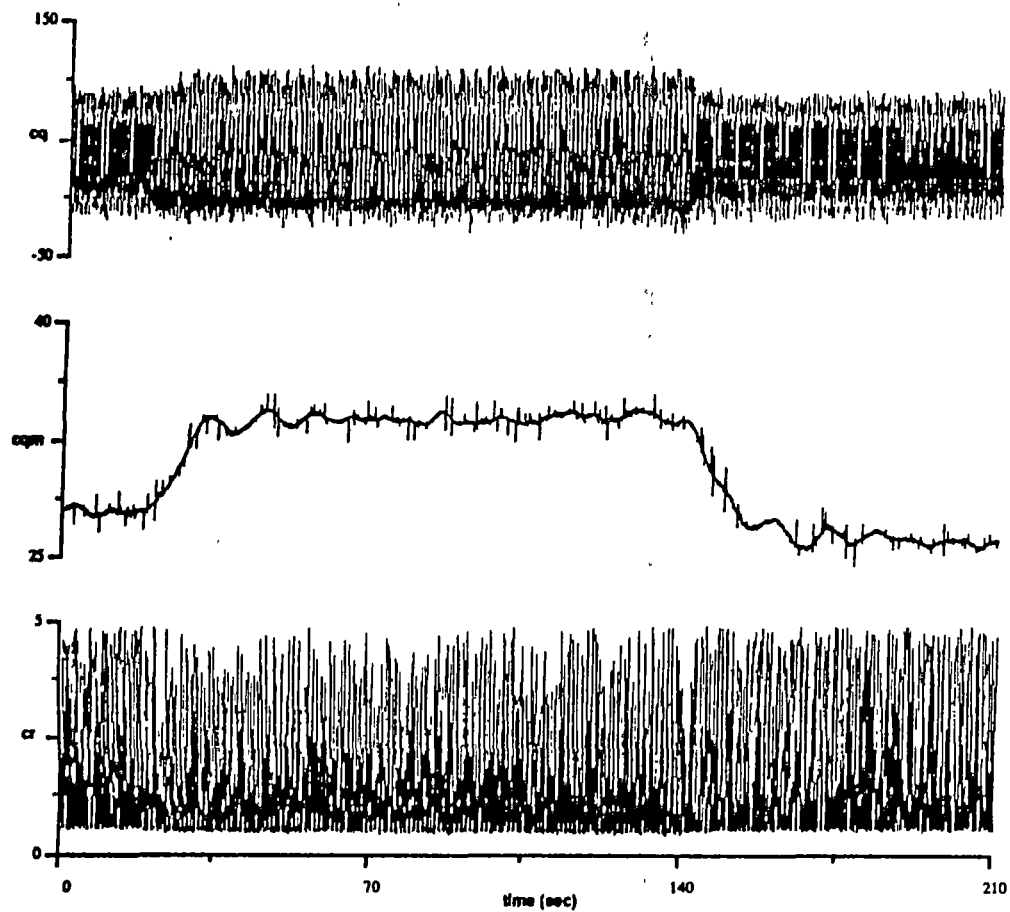
<sup>1</sup>Using programme *mndst* (sec section 4.1.2.5).

<sup>2</sup>Same symbols were used as Figure 4.6.





**Fig. 4.7:** The changes of proximal aortic pressure due to a gradual aortic constriction



1 second

Fig. 4.8(Aa): Hemodynamic variables before, during and after aortic constriction. (cq - coronary blood flow; cqm - mean coronary blood flow; cr - calculated coronary resistance). continued on next page.

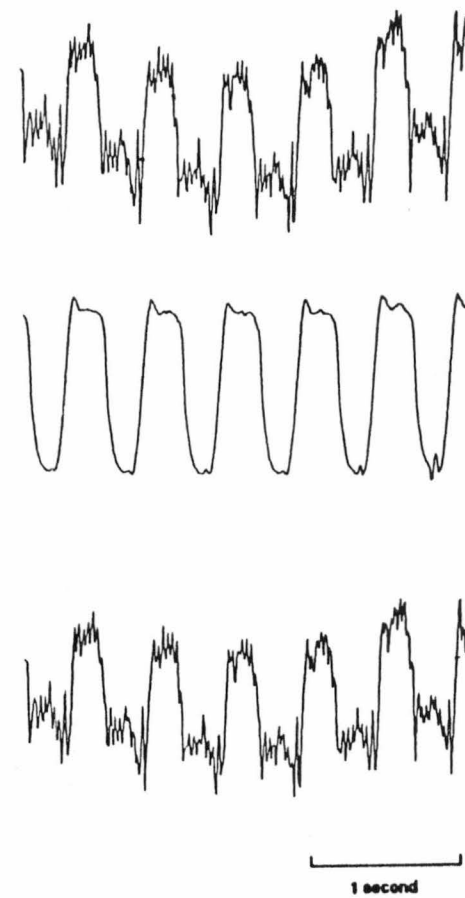
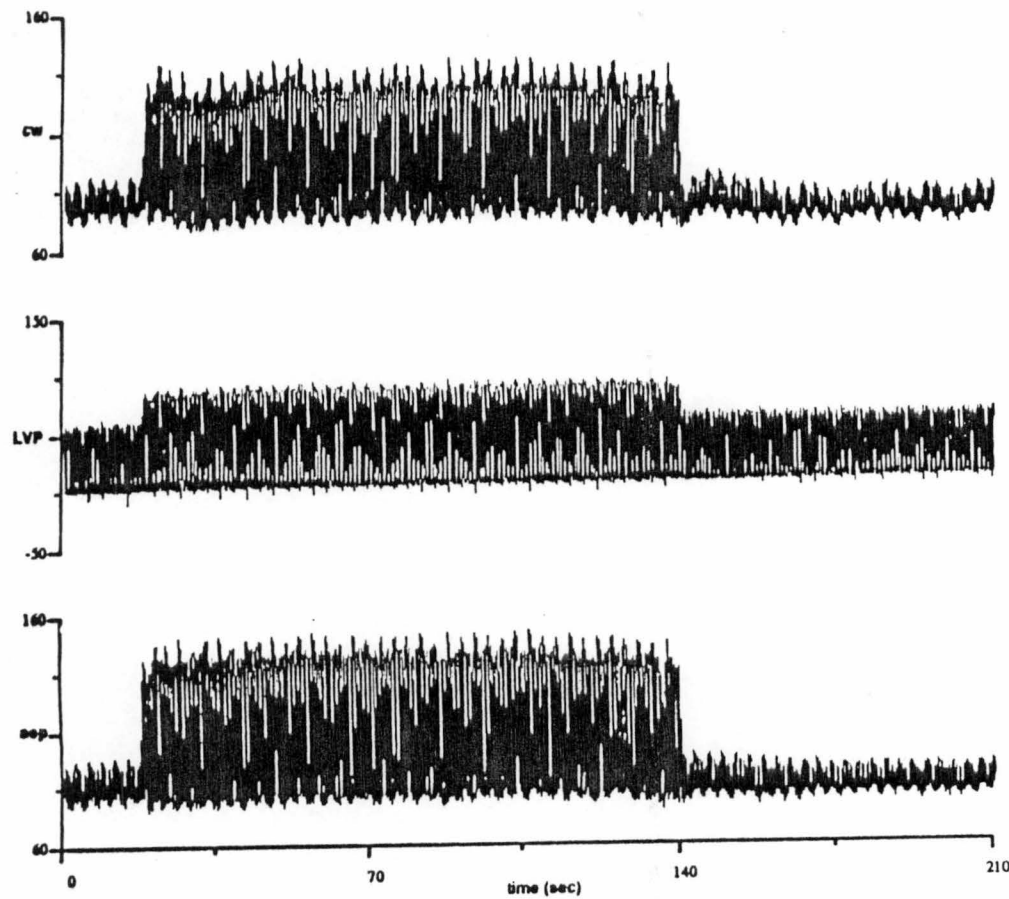
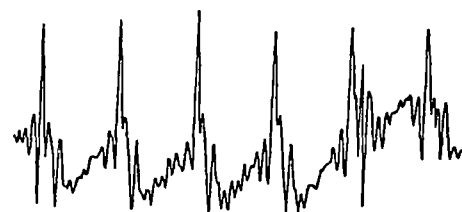
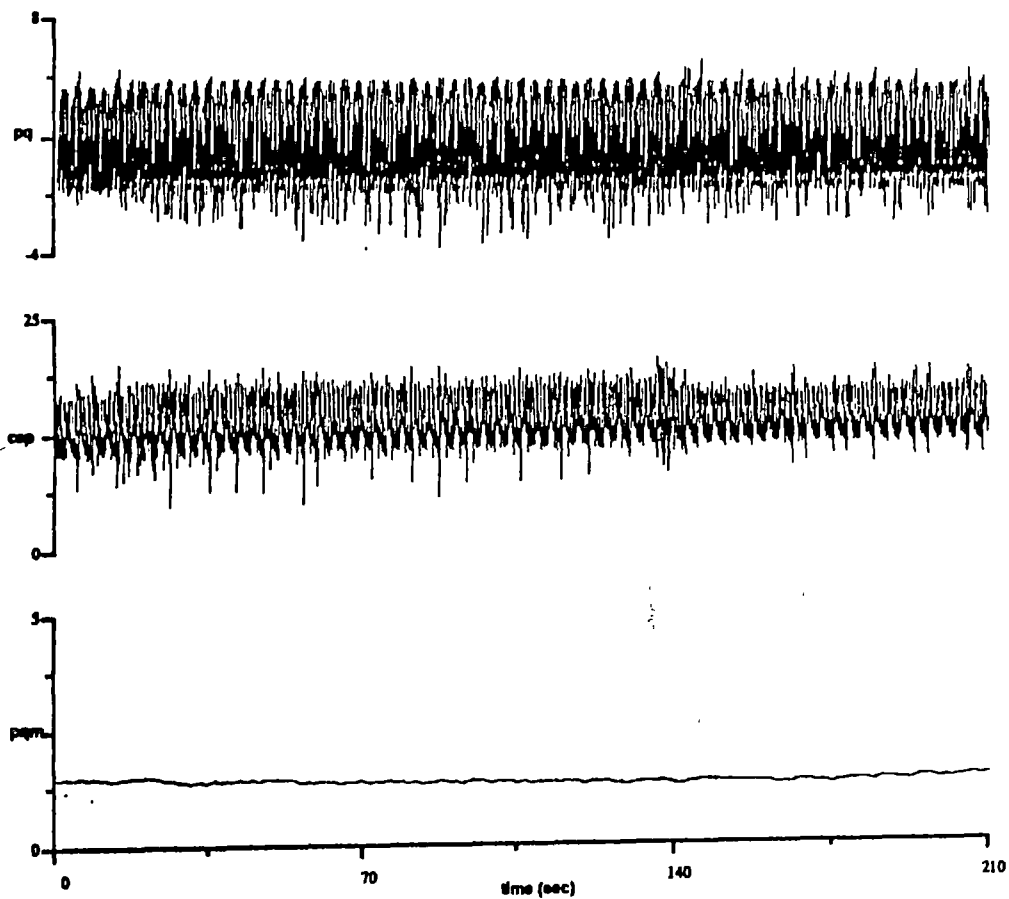
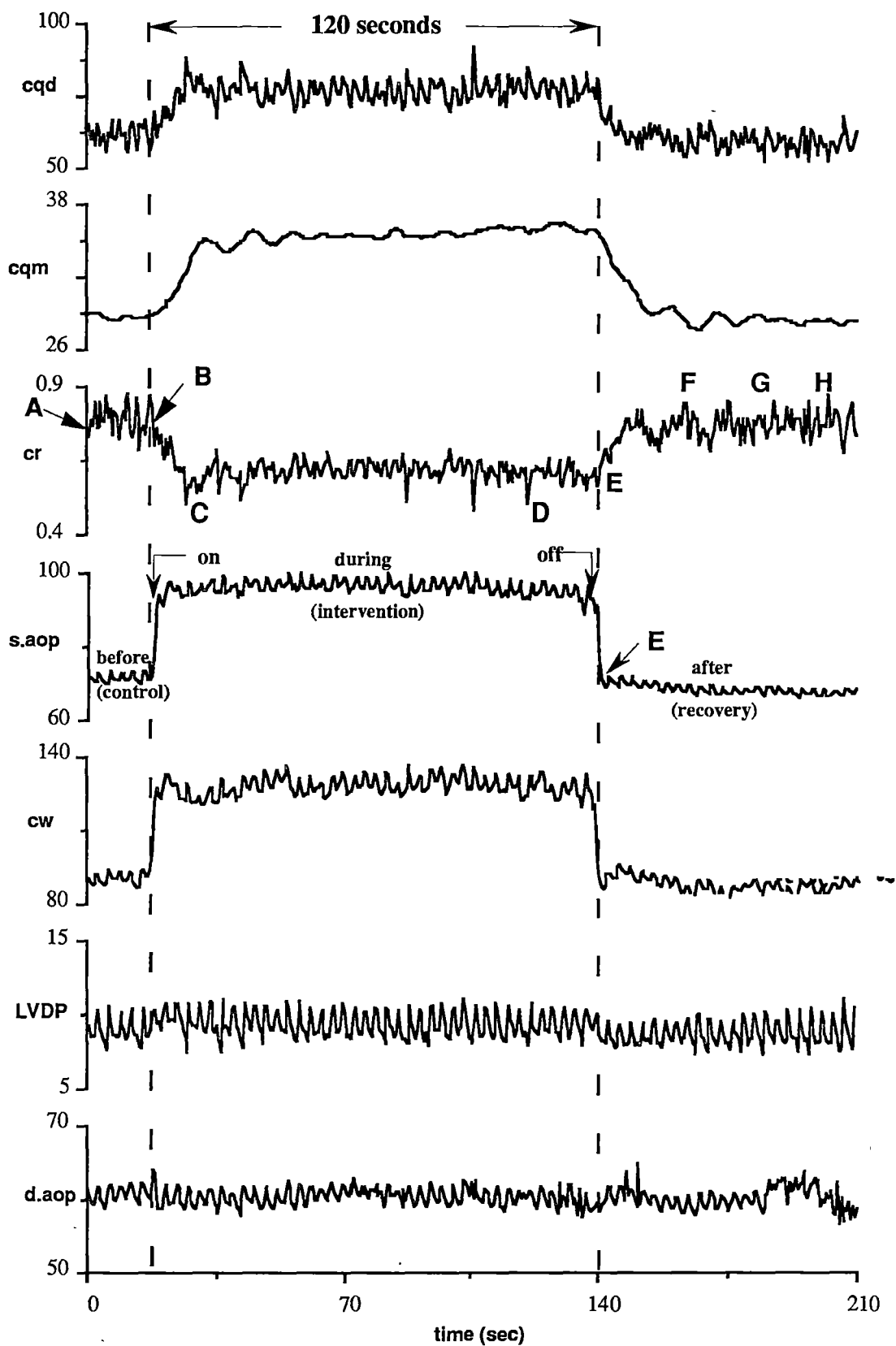


Fig. 4.8(Ab): Hemodynamic variables before, during and after aortic constriction. (cw - calculated cardiac work; LVP - left ventricular pressure; aop - proximal aortic pressure). continued on next page.



1 second

**Fig. 4.8(Ac):** Hemodynamic variables before, during and after aortic constriction. (pq - pulmonary artery flow; csp - coronary sinus pressure; pqm - mean pulmonary artery blood flow).



**Fig. 4.8(B):** Mean diastolic and systolic hemodynamic continuous changes before, during and after aortic constrictions. (see text for details)

In Figure 4.8(B), **cqm** and **cqd** increases and **cr** decreases following the cardiac work increases induced by proximal aortic constriction. This figure shows the response immediately after intervention, then **CBF** and **cr** stabilizing until the intervention is withdrawn. Immediately thereafter, **CBF** and **cr** return toward the baseline before intervention. This is called the offset response or decay in response to the withdrawal of the intervention. Due to the many factors which might affect the onset data, only offset responses were analysed. The programme *xu4*<sup>1</sup> was used to select the offset response starting point E in Figure 4.8(B). E-F-G-H represents the decay of the response after the withdrawal of intervention. This section of the response was fitted to an exponential decay<sup>2</sup> and half lives were obtained. Figure 4.9, 4.10 and 4.11 represent the curve fitting for the control, adenosine blocking, and adenosine,  $\alpha$ ,  $\beta$  receptor blocking data.

Linear regressions were calculated for the calculated decays against the degree and duration of aortic constrictions. The relationships between the  $t_{1/2}$  and the percentage change of **cqm**, **cqd** and **cr** were also determined. Peak change of the **cqm**, **cqd** and **cr** was read directly by computer programme and was expressed as a percentage of control. Statistical analysis is the same as described in section 4.3.4.

#### 4.4.2 Actual Protocol for Chapter Six

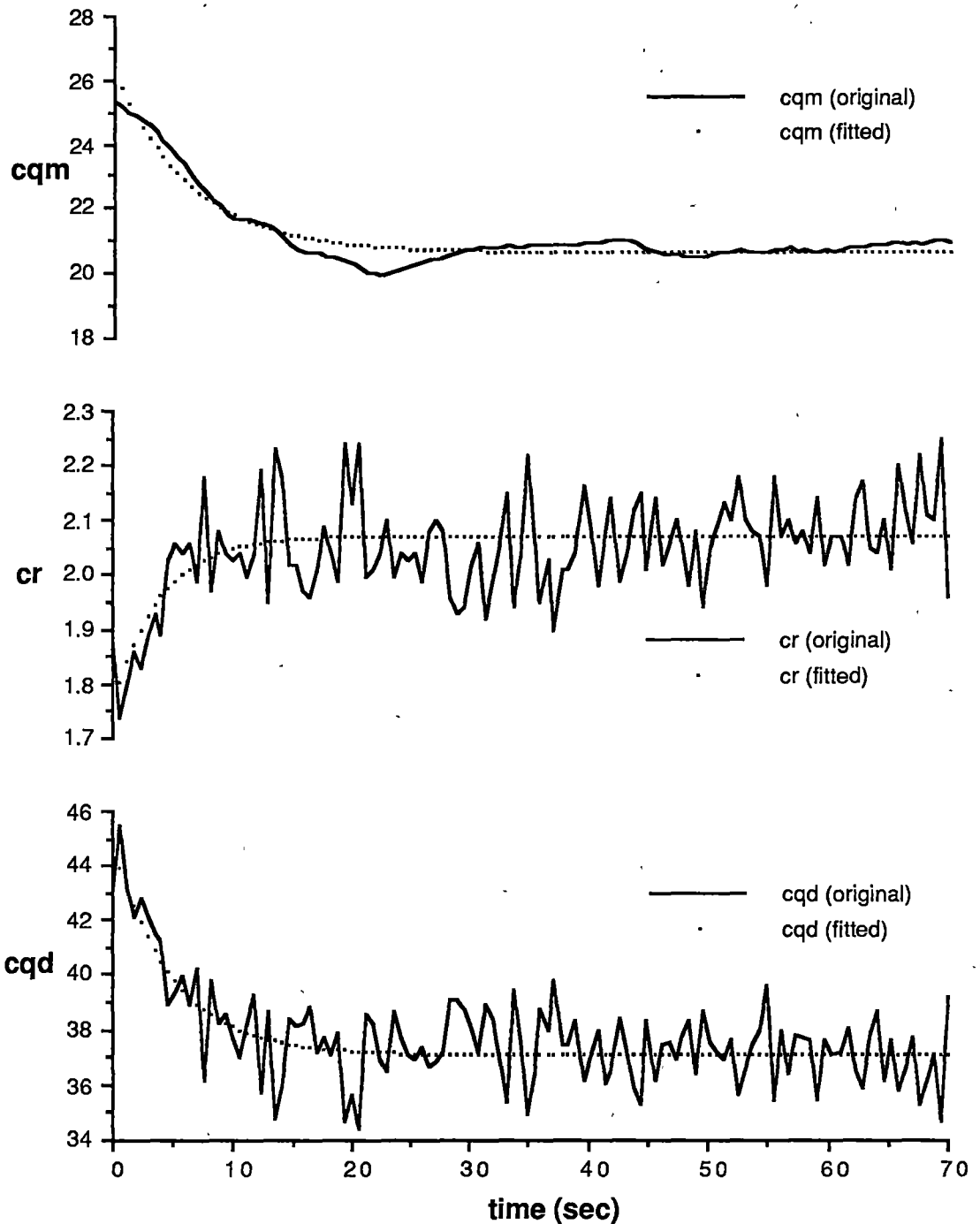
##### 4.4.2.1 Aims

The aim of this group of experiments was to study the relationship between coronary resistance and metabolic parameters in response to the different degrees of systemic hypoxia before and after  $\alpha$ ,  $\beta$  and adenosine receptor inhibitions.

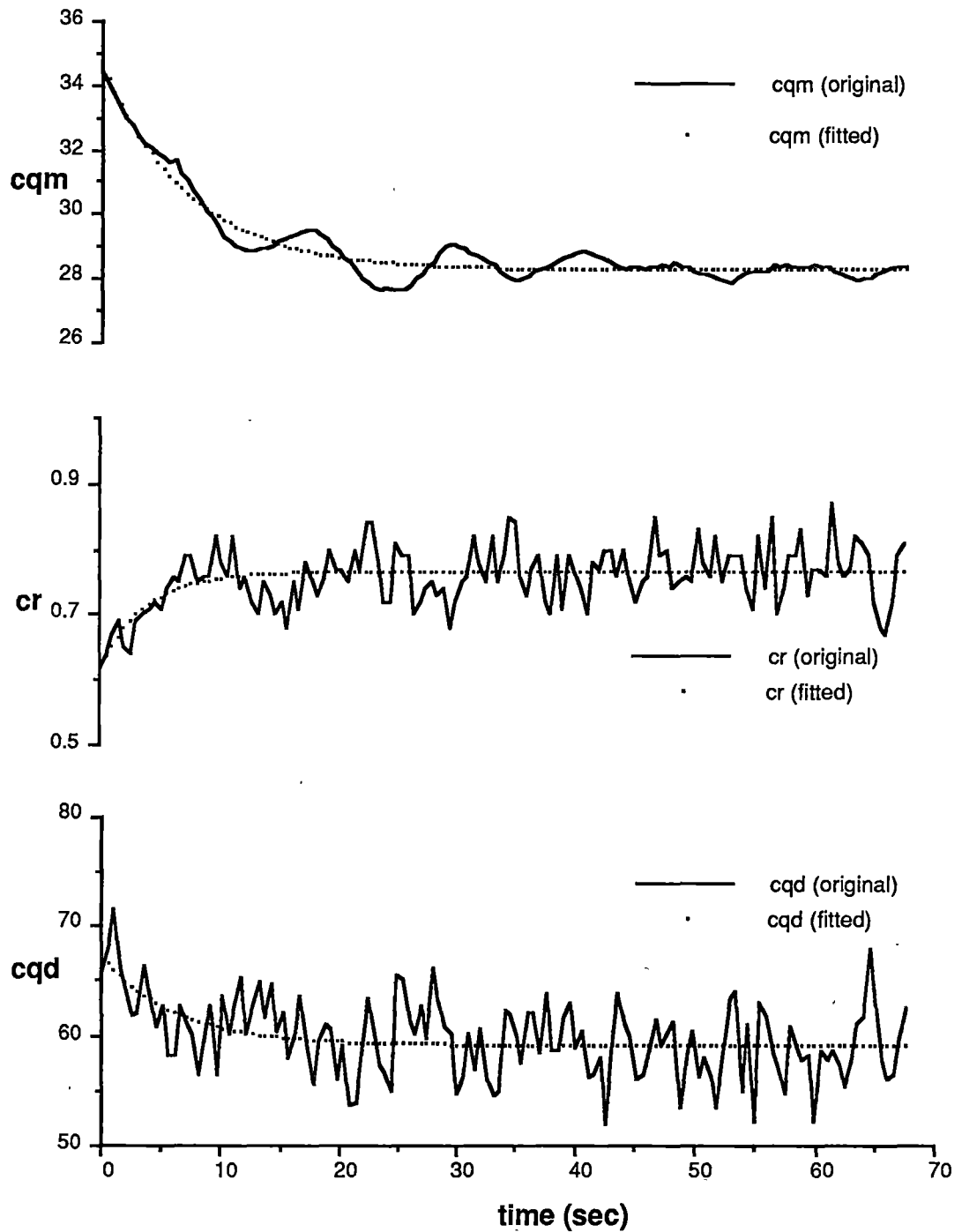
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<sup>1</sup>Refer to section 4.1.

<sup>2</sup>Using programme *congrad* and equations 4.4 and 4.5 (refer to section 4.1.2.5 and 4.3.2.2)

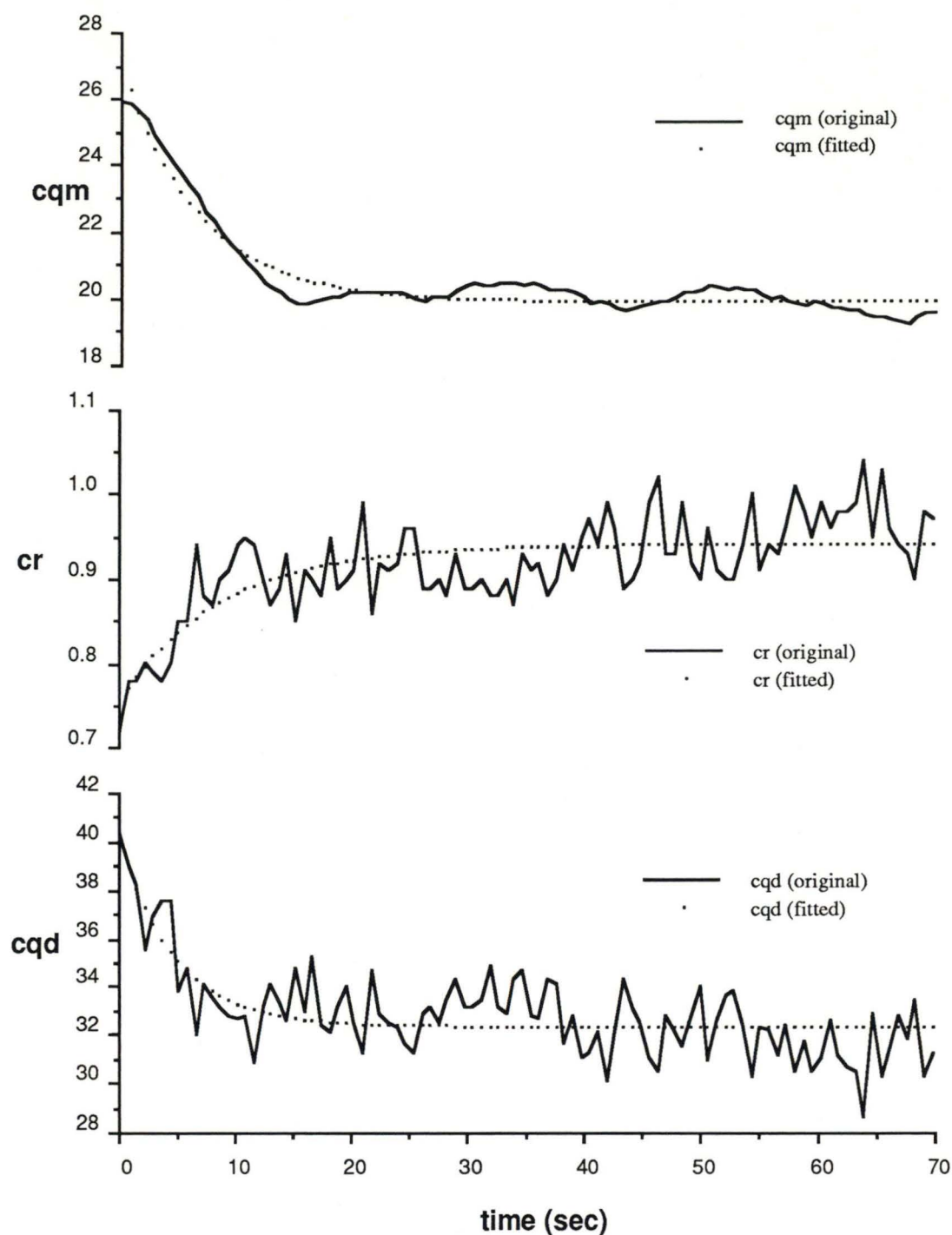


**Fig.4.9:** Exponential curve fitting of decay in cqm,cr and d.cq in control data of group 2. cqm - mean coronary blood flow; cr - diastolic mean calculated coronary resistance; cq - diastolic mean coronary blood flow.  $t_1(\text{cqm}) = 5.12$ ;  $t_1(\text{cr}) = 5.33$ ;  $t_1(\text{cq}) = 5.06$  (data from experiment 90.10.8, increase of proximal aortic pressure is 28%; increase of calculated cardiac work is 30%; duration of intervention is 120 secs. 128 data points)



**Fig.4.10:** Exponential curve fitting for cqm, cr and cqd after adenosine blockade. cqm, mean coronary blood flow; cr, diastolic mean calculated coronary resistance; cqd, diastolic mean coronary blood flow.  $t_{1/2}(cqm) = 4.93$ ;  $t_{1/2}(cr) = 3.85$ ;  $t_{1/2}(cqd) = 4.03$ . (Data from experiment 90.9.25, increase of proximal aortic pressure is 46%; increase of calculated cardiac work is 44%; duration of intervention is 120 seconds. 137 data points)





**Fig.4.11:** Exponential curve fitting after adenosine,  $\alpha$  and  $\beta$  receptors blockage. **cqm** - mean coronary blood flow; **cr** - diastolic mean calculated coronary resistance; **cqd** - diastolic mean coronary blood flow. For these data,  $t_{1/2}(\text{cqm}) = 5.96$ ;  $t_{1/2}(\text{cr}) = 5.49$ ;  $t_{1/2}(\text{cqd}) = 3.68$ . (data from experiment 90.11.12, increase of proximal aortic pressure is 50%; increase of calculated cardiac work is 37%; duration of intervention is 120 secs. 97 data points)

#### 4.4.2.2 Methods

Table 4.4 shows number of sheep for different parts of the experiment.

**Table 4.4:** Number of sheep used in experimental work described in this chapter

	<b>control (group1)</b>	<b>inhibition (group2)</b>		
		<b>control</b>	<b>adenosine inhibition</b>	<b>adenosine+<math>\alpha</math>-, <math>\beta</math>-adrenergic inhibitions</b>
No. of sheep	10	8	8	8
successful experiments	8	6	6	5

##### *Group 1*

This was a control group in which no blockers were used. It consisted of 10 sheep. 8 animals were successful and two died. A similar protocol to that used for chapter 5 was performed except hypoxia was the intervention. Each gas mixture was used for ventilation for different durations causing different degrees of systemic hypoxia. Experimental runs were randomized and each set required at least 3 runs per sheep. An interval of at least 20 minutes was allowed for animal to recover completely between runs, which means all the hemodynamic variables and blood gas analysis had reached a stable value.

##### *Group 2*

There were 6 successful experiments in this group. Sheep were instrumented with intracoronary cannulation for intracoronary adenosine injection in addition to the basic setup as group 1 for determination of the **8-PT** blocking effect. Similar procedures were carried out as described for group2 of chapter 5 except systemic hypoxia was a stimulus.

#### 4.4.2.3 Data Analysis

Figure 4.12 shows the hemodynamic variables in response to hypoxia on the chart - recording. Figure 4.13(B) demonstrates the mean diastolic and systolic data<sup>1</sup> from digitized and calibrated data shown in Figure 4.13(Aa,b,c) on a beat by beat basis. The onset and offset of CBF and cr due to systemic hypoxia can be seen. In Figure 4.13<sup>2</sup>, systemic hypoxia was caused by inspiring type I gases and lasted 120seconds. This caused the proximal aortic pO<sub>2</sub> to reach 35.9mmHg and SO<sub>2</sub> 74.6%, 490seconds of data (992beats) were digitized. It included the data before intervention A-B, during intervention D-E (120secs) and after intervention E-E'-F-G-H. MVO<sub>2</sub> was calculated by averaging cqm (A-B) and picking up highest value (I) by programme *expfit* and using equation 4.3, then both baseline MVO<sub>2</sub> and hypoxia MVO<sub>2</sub> were obtained.

Because there were concomitant changes including pressure, flow and metabolic parameters due to systemic hypoxia, it was prohibited to study the decay of the response after the withdrawal of intervention. The analysis has directed to study the relationships amongst the coronary resistance, metabolic parameters and MVO<sub>2</sub> before and after adenosine,  $\alpha$ ,  $\beta$  receptor blockades.

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<sup>1</sup>Calculated by *mindst* .

<sup>2</sup>Same symbols and abbreviations were used as in Figures 4.6, 4.7 and 4.8.

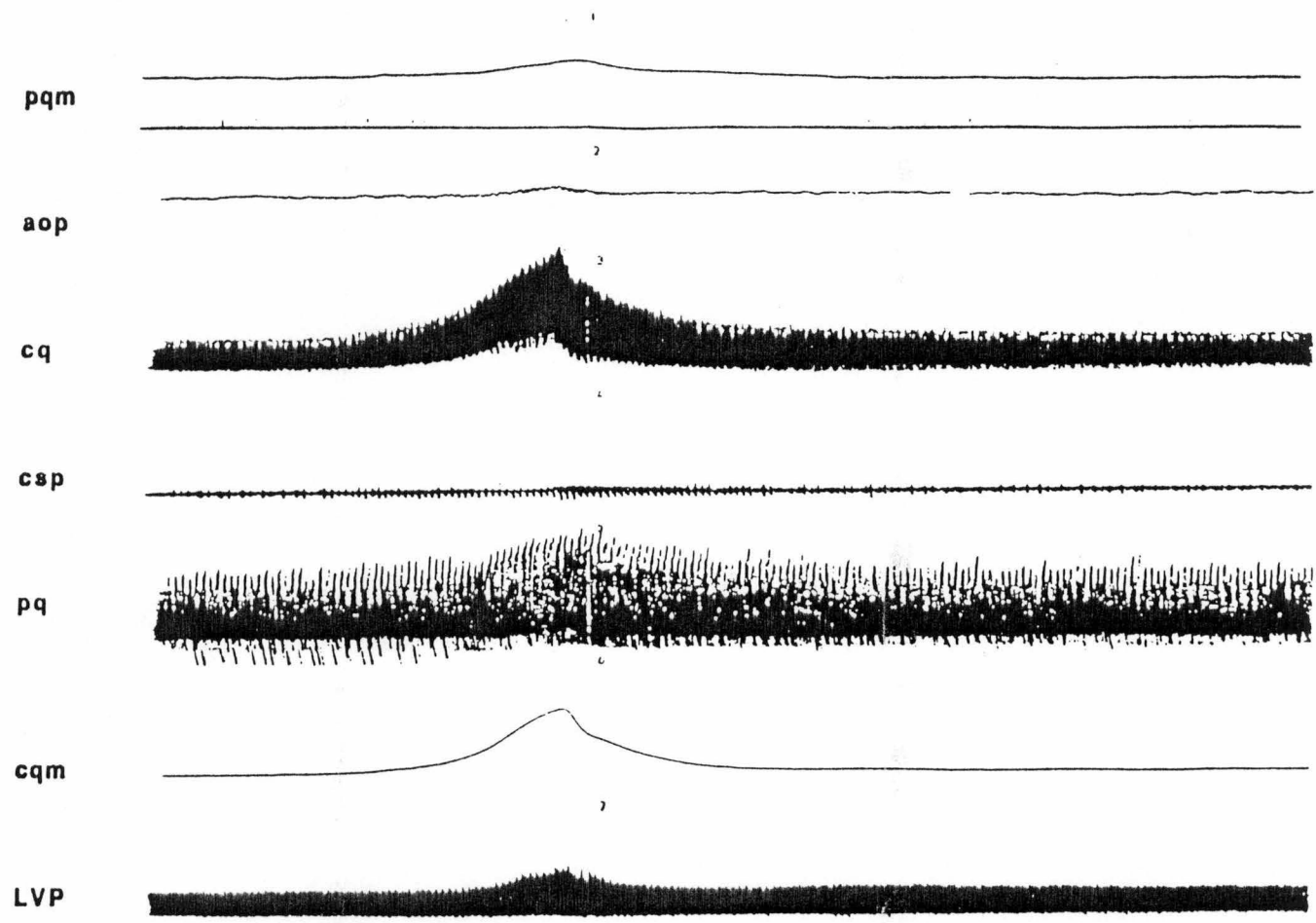


Fig. 4.12: The changes of hemodynamic variables due to systemic hypoxia in chart-recording

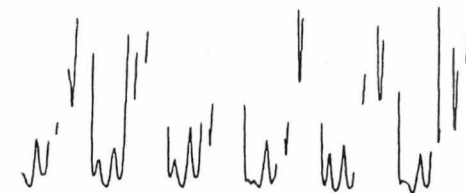
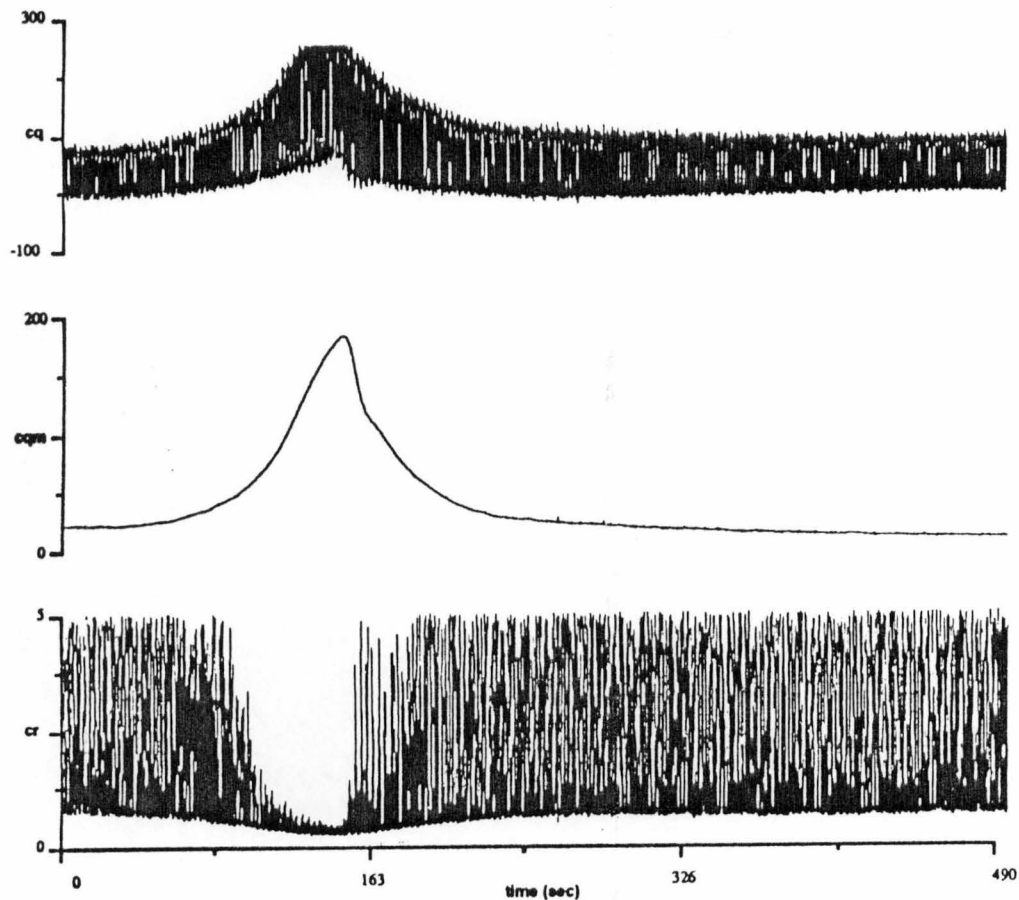


Fig. 4.13(Aa): Hemodynamic variables before, during and after systemic hypoxia. (cq - coronary blood flow; cqm - mean coronary blood flow; cr - calculated coronary resistance). continued on next page.

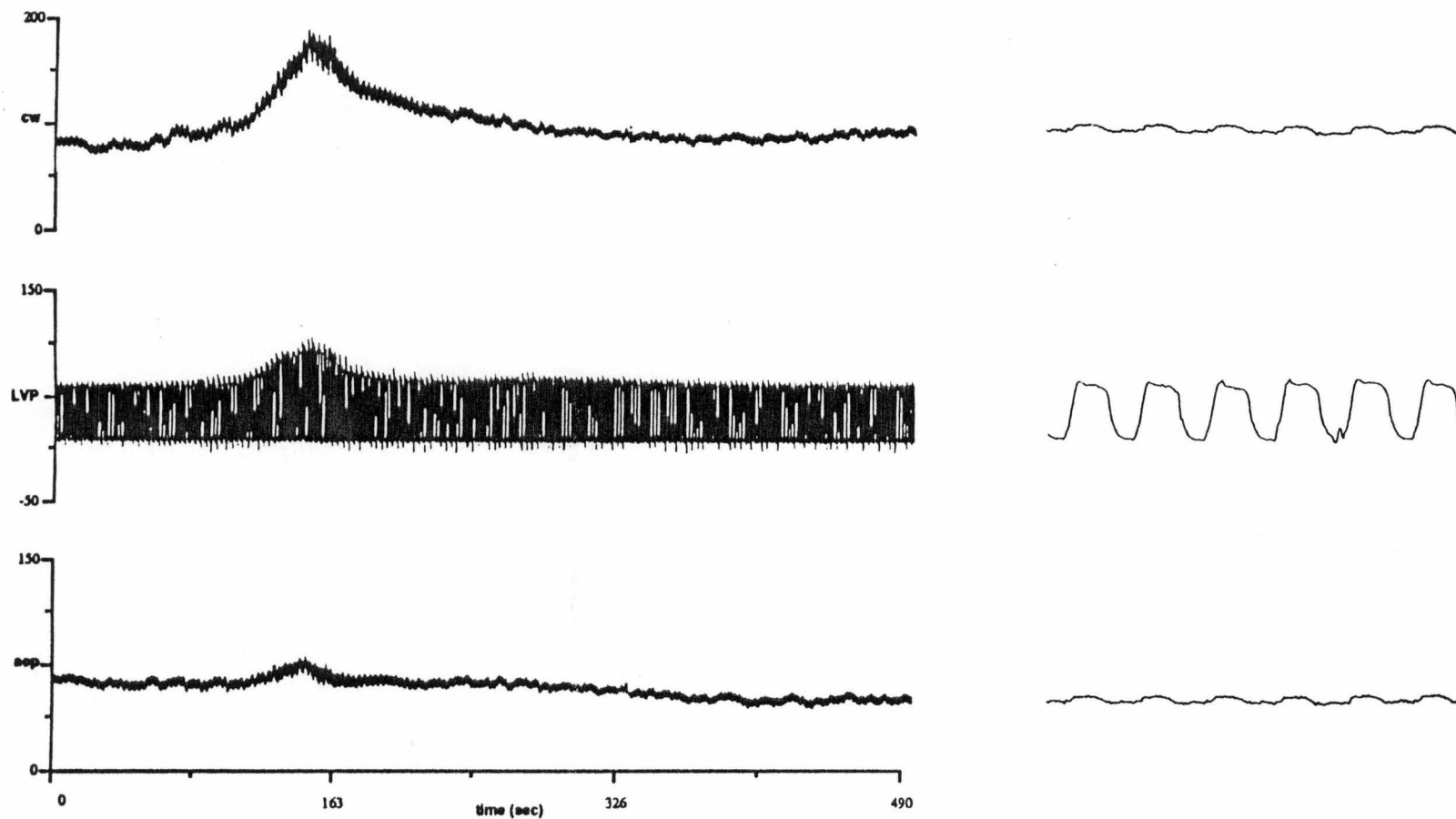


Fig. 4.13(Ab): Hemodynamic variables before, during and after systemic hypoxia. (cw - calculated cardiac work; LVP - left ventricular pressure; aop - proximal aortic pressure). continued on next page.

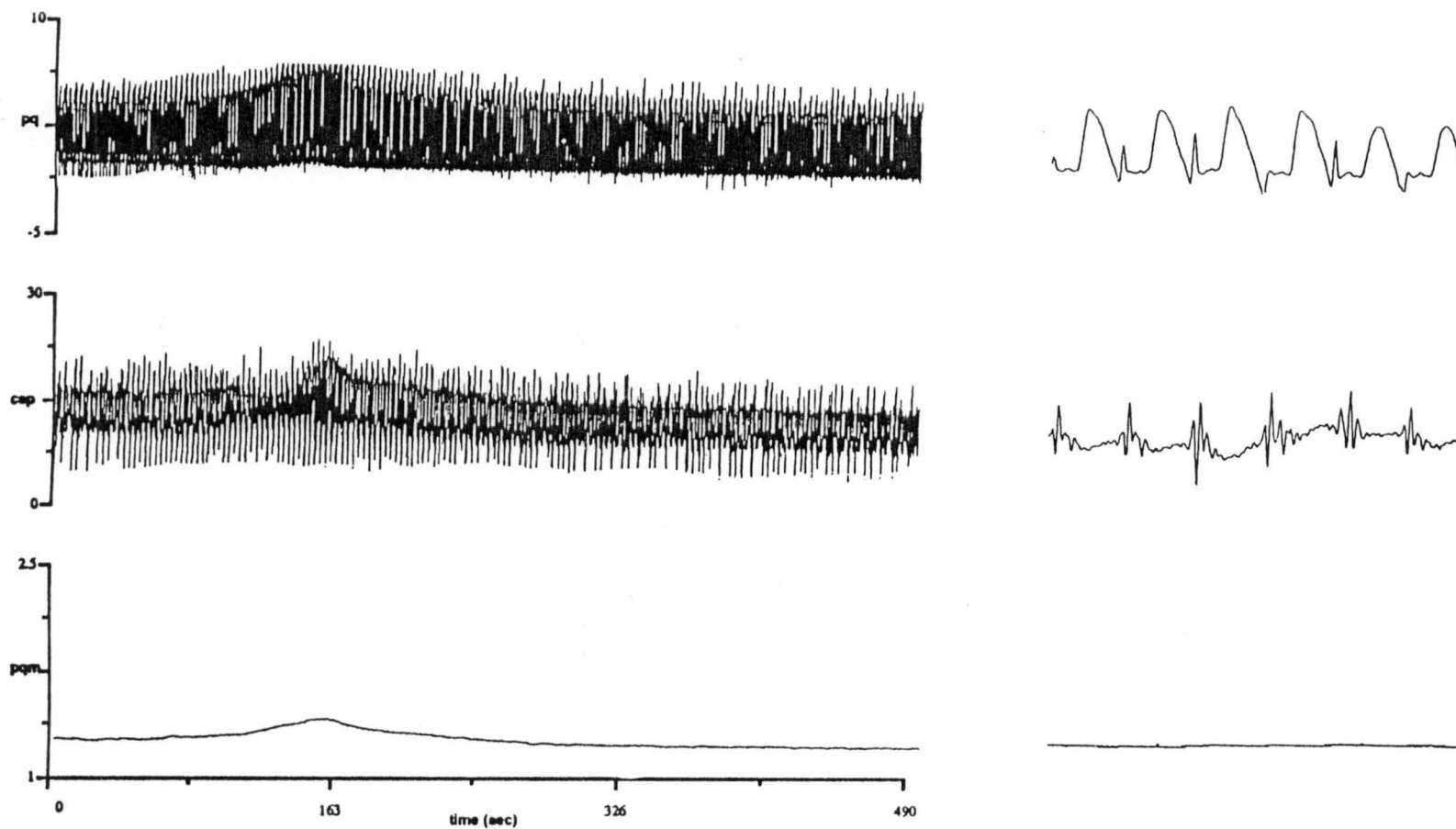
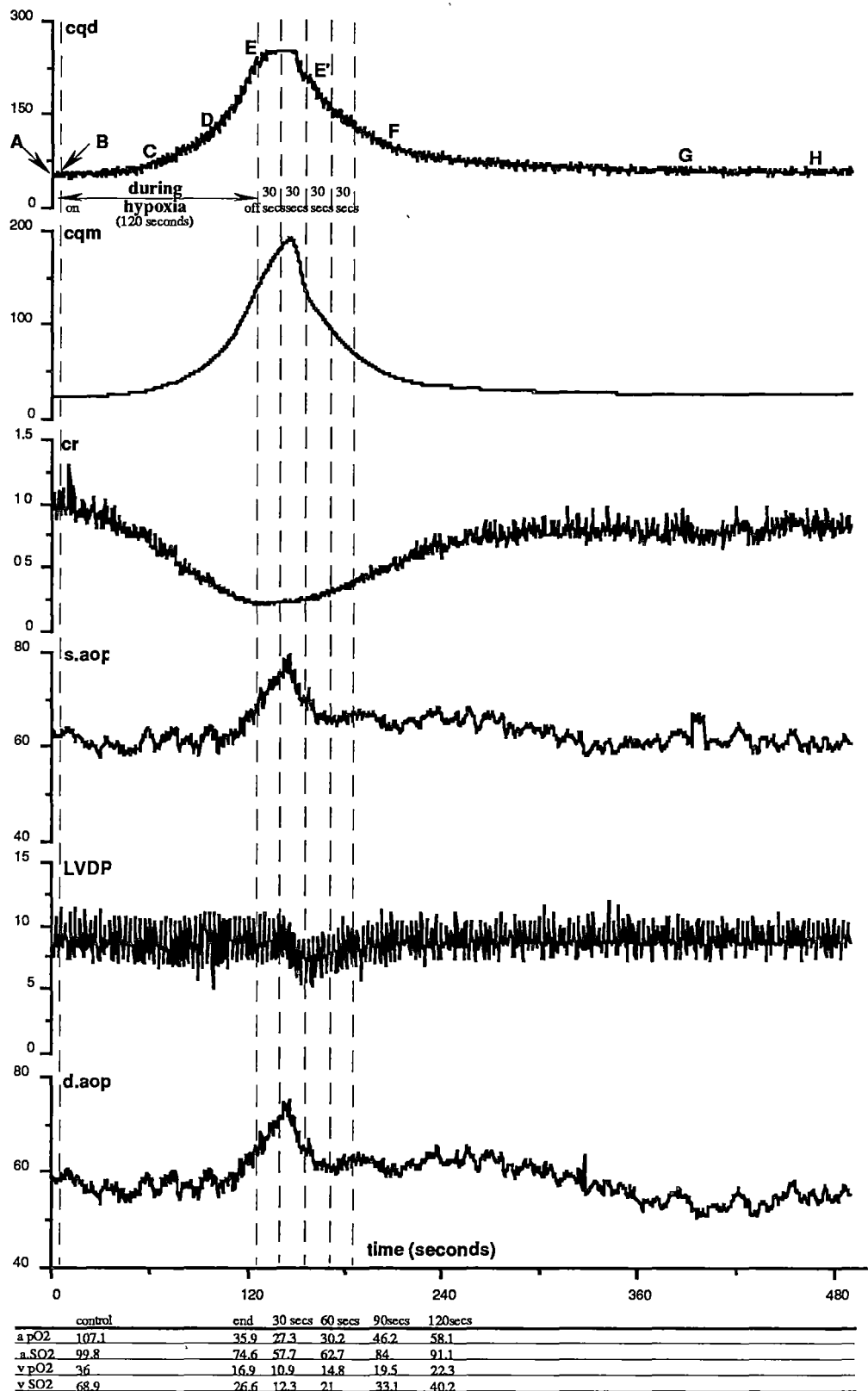


Fig. 4.13(Ac): Hemodynamic variables before, during and after systemic hypoxia. (pq - pulmonary artery flow; csp - coronary sinus pressure; pqm - mean pulmonary artery blood flow).



**Fig. 4.13(B):** Mean diastolic and systolic hemodynamic continuous changes before, during and after systemic hypoxia. (see text for details)



### **4.4.3 Actual Protocol for Chapter Seven**

#### **4.4.3.1 Aims**

The aim of this experiments was to study the relationship between the coronary sinus (CS) O<sub>2</sub> content (SO<sub>2</sub>) and both the increased cardiac work and systemic hypoxia and to determine which feedback control system, the 'open-loop' or 'close-loop', operates in coronary circulation. In other words, I wanted to investigate whether metabolic vasodilation exists solely for supplying blood for the oxygen needs of the heart during increased cardiac work and systemic hypoxia.

#### **4.4.3.2 Methods**

The same experimental sheep and setup as in chapter 5 and chapter 6 were used for this investigation. An OXIMETRIX fibre optic catheter was inserted into CS for continuous measurement of CS SO<sub>2</sub>. Blood samples were taken from both proximal aorta and CS simultaneously for pO<sub>2</sub> and other blood gases and acid-base balance assessment. In this group, seven sheep were used.

#### **4.4.3.3 Data Analysis**

Data analysis followed the procedures described in sections 4.3.3 and 4.3.4.

### **4.4.4 Actual Method for Chapter Eight**

#### **4.4.4.1 Aims**

The aim of this final group experiments was to determine the dose-response curve of intracoronary infusion of adenosine and the decay ( $t_{1/2}$ ) of CBF and cr in response to intracoronary exogenous adenosine infusion. 8-PT blocking effect of exogenous adenosine is also observed.

#### 4.4.4.2 Methods

Apart from the basic setup described previously, polyethylene tubing<sup>1</sup> (OD: 0.61mm; ID: 0.28mm; PE: 10) was inserted into the coronary circumflex artery as coronary cannulation before the flowmeter probe for intracoronary infusion (see Figure 4.14). 10 sheep were included in this experiment. The response of CBF to

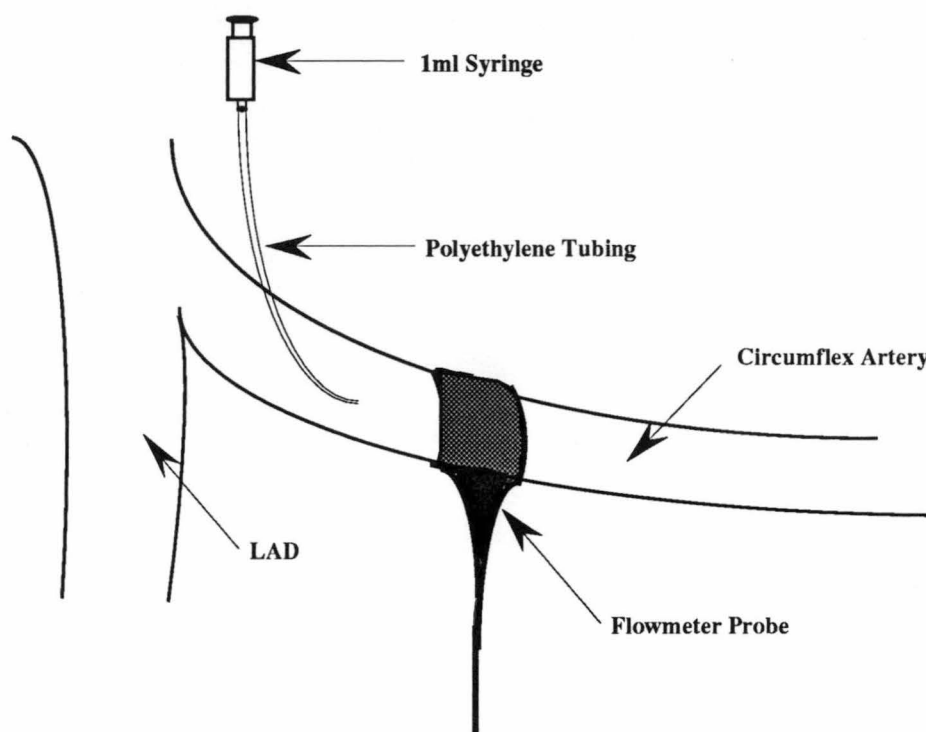


Fig. 4.14: Diagrammatic representation of the intracoronary cannulation

intracoronary bolus administration of adenosine in constant volume doses containing of 0, 2, 5, 10, 20, 50, 100 and 200 $\mu$ g were observed. In each case, the adenosine was dissolved in 0.25 ml of normal saline. Between each dose, the sheep were allowed to recover fully.

Figure 4.15 illustrates the response of **cqm**, **cqd**, **cr**, **s.aop**, **LVDP** and **d.aop** to exogenous adenosine (10 $\mu$ g). The highest value (point I) was picked up by

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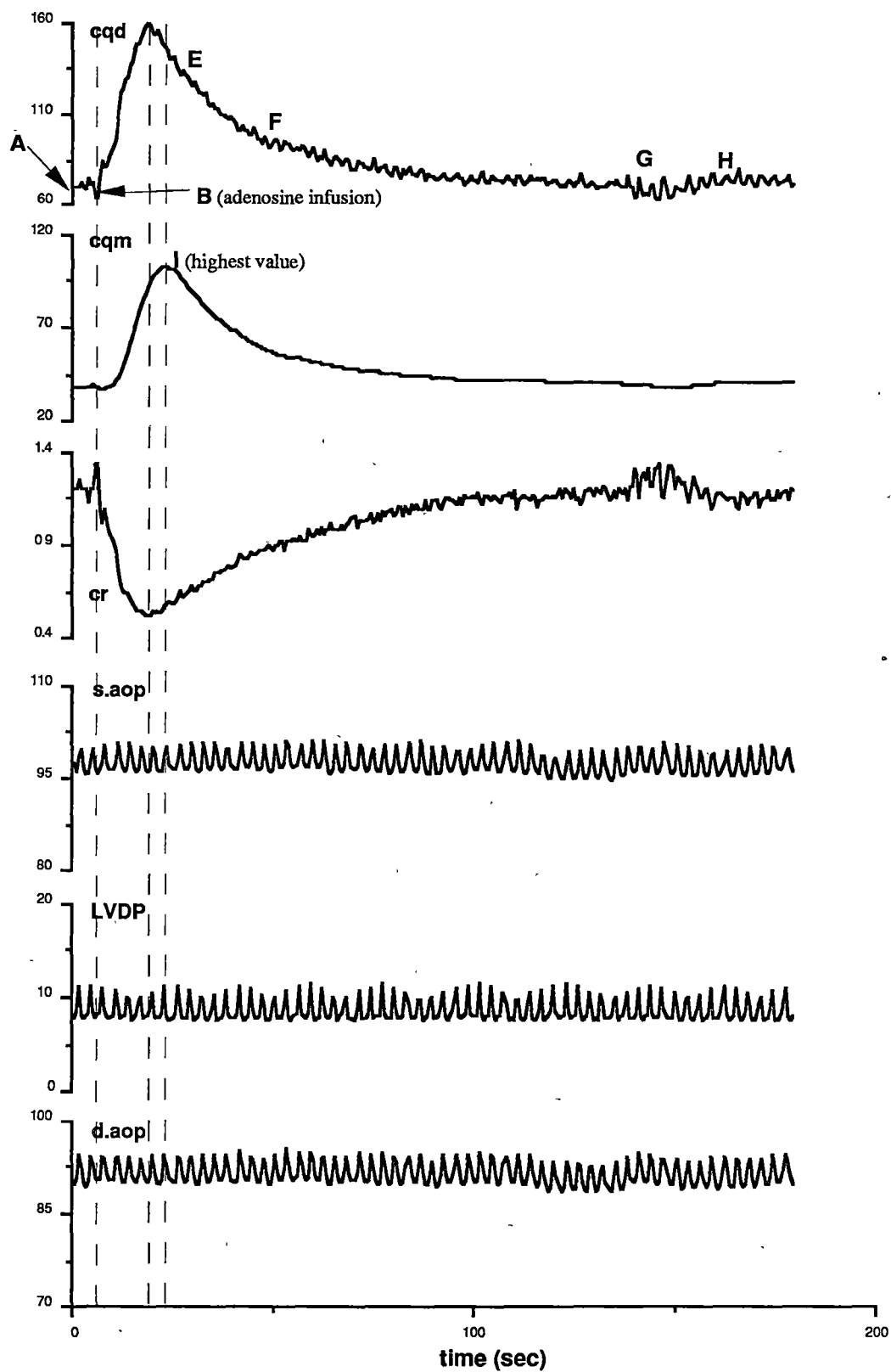
<sup>1</sup>Supplied by Dural Plastics and Engineering, Dural, N.S.W., 2158, Australia

the programme *expfit*, and the E' point also determined. Thereafter, the data pieces in the region E'-F-G-H were used by the programme *congrad* for exponential curve fitting. The values obtained are shown in Figure 4.16.

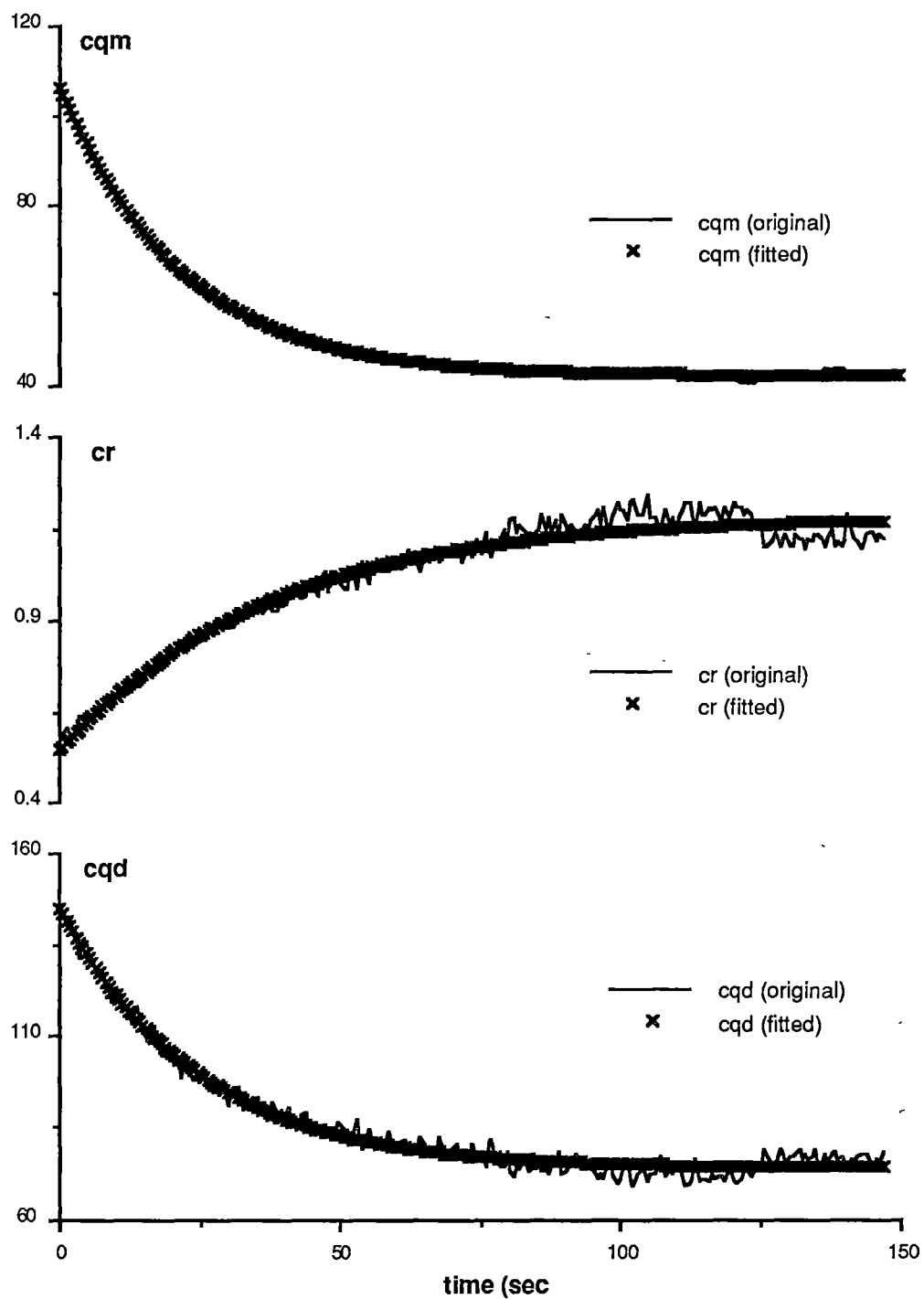
After giving 8-PT adenosine dose-response curves were obtained to determine the blocking effect. Half lives of exogenous adenosine after blockade were also calculated by the same procedure.

#### **4.4.4.3 Data Analysis**

The procedures already described in sections 4.3.3 and 4.3.4 were used.



**Fig. 4.15:** Mean diastolic and systolic hemodynamic continuous changes before and after intracoronary adenosine infusion (10 $\mu$ g). (see text for details)



**Fig.4.16:** Exponential curve fitting after exogenous adenosine 20 $\mu$ g infusion (255 data points):

$$t_{1/2}(cqm) = 14.6; \quad t_{1/2}(cr) = 25.0; \quad t_{1/2}(cqd) = 16.7.$$

## **4.5 DISCUSSION OF TECHNIQUES**

### **4.5.1 Discussion of Experimental Methods**

#### **4.5.1.1 Sheep as the Experimental Animal**

As far as fundamental physiological mechanisms are concerned, if differences between human and higher mammals exist, they often seem to be of a more quantitative than qualitative nature. Therefore, it is reasonable to use the acute sheep model. The sheep, because of its coronary venous system anatomy, is a suitable model for this work. However, this work will need to be repeated in another animal model.

#### **4.5.1.2 Intervention – stenosis**

The aortic band enables us to force the sheep heart to a high performance state by suddenly constricting the aorta. If the sheep was well prepared and all the parameters were stable, it was found that this method only increased systolic pressure (afterload on the heart), whereas the proximal aortic diastolic pressure remained the same, indicating that this intervention does not change the coronary driving pressure since the coronary artery flow is mainly determined by diastolic pressure [Katz, 1988]. In addition, this method enabled the aortic cross-sectional area to be changed in the desired range. Again, if the sheep's condition was good, it was possible to get a repeatable alteration of aortic pressure by constricting to the same aortic cross-sectional area.

Braunwald [1958] demonstrated that aortic constriction increased  $\dot{MVO}_2$ , but reduced (A-V)  $O_2$ . However, Blumenthal and co-workers [1962] found that aortic stenosis distal to the coronary ostia lowered (A-V)  $O_2$  but stenosis proximal to the ostia raised myocardial oxygen extraction. This was further supported by Saito and his colleagues [1982], who showed that constriction of the descending thoracic aorta

increased  $\text{MVO}_2$ , but reduced myocardial oxygen extraction in open-chest dog hearts. This reduction in (A-V)  $\text{O}_2$  was abolished by atropine or vagotomy. In the current work, an aortic band was placed proximal to the coronary ostia, it suggested there was no parasympathetic coronary vasodilation involved as far as the (A-V) $\text{O}_2$  was concerned. This led to an experimental design without using any parasympathetic blocking interferences.

The change of aortic pressure may affect the left ventricular pressure. Left ventricular diastolic pressure influences on the diastolic coronary pressure-flow relationship have been reported [Aversano, 1984]. In addition, aortic stenosis elicits a similar increase in left ventricular systolic pressure which will compresses intramyocardial vessels and result in a retrograde pressure wave [Spaan, 1985], increasing coronary resistance and decreasing mean CBF [Sabiston, 1957]. However, with the servo-controlled coronary arterial pressure system, systolic contraction did not significantly impede diastolic CBF at heart rates up to 160beats/min by comparing flow during diastole with flow during a prolonged asystole [Katz, 1988]. This confirmed that diastolic CBF is not influenced by systole under normal circumstances. However, if the left ventricular diastolic pressure is not changed, which is the case in this work, diastolic intramyocardial vascular waterfall may be absent although conclusive proof for the presence or absence of intramyocardial waterfall phenomena is not yet available. Therefore, diastolic CBF was separated for data analysis to exclude the influence of ventricular systolic pressure on the mean CBF in this work.

Gregg [1963] found the perfusion pressure for coronary arteries to be similar to aortic ones and that left CBF was predominant in diastole in dogs. A close linear relationship between pressure and flow was demonstrated by Bellamy [1978] during diastole. It is logical to believe that coronary perfusion pressure is similar to aortic diastolic pressure. Thus, it is important to exclude the effect of aortic constriction on

the diastolic aortic pressure. Therefore the criterion, aortic diastolic pressure does not change significantly in response to aortic constriction, was used for data selection. This matches most of my experiments, which may be because diastolic pressure was mainly determined by the peripheral circulation. The data in Figure 4.8 demonstrate that there was no significant effect of aortic constriction on diastolic aortic pressure.

In addition, careful examinations of all hemodynamic variables and some indices of myocardial metabolism (table 5.1) ensured that heart rate and diastolic left ventricular pressure did not contribute to the change of cardiac work. Since there was also no alteration of pulmonary artery flow, it is reasonable to use the increase of aortic pressure as an estimate of increased cardiac work.

In summary, the proximal aortic constriction<sup>1</sup> did not cause the significant changes in diastolic proximal aortic pressure, diastolic left ventricular pressure, heart rate, cardiac output. However, the formula used to calculate the coronary resistance can also minimize those pressure effects. It is reasonable to use this stimulus to study the decay of the local coronary vasoregulation in response to the increased cardiac work.

#### **4.5.1.3 Intervention – systemic hypoxia**

Three types of gas mixtures were used to produce systemic hypoxia artificially on the sheep. If the sheep condition was stable, repeatable systemic hypoxia could be induced by adjusting the gas supply.

#### **4.5.1.4 The Selections of Adenosine, $\alpha$ - and $\beta$ -Receptor Blockades**

So far, many agents have been found to have blocking action on the adenosine induced vasodilation such as dipyridamole, aminophylline, theophylline and adenosine

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<sup>1</sup>This stimulus was applied for no more than 3 minutes.



deaminase<sup>1</sup>. However, the competitive inhibition of adenosine by methylxanthine was incomplete, for instance,  $10^{-6}$  M adenosine which is a concentration that produced 85% of maximal coronary dilation can only be inhibited by 20% by  $5.5 \times 10^{-5}$  M theophylline [Bünger, 1975; Merrill, 1978]. 8-phenyltheophylline (**8-PT**) was found to be the most potent available adenosine antagonist with relatively low activity of phosphodiesterase inhibition [Smellie, 1979]. Thus, **8-PT** was chosen as adenosine inhibitor in this investigation. The effectiveness of adenosine blockade was showed by the comparison of the effect of adenosine vasodilation during intracoronary bolus adenosine injection before and after systemic administration of **8-PT**.

Phentolamine and propranolol are the classic  $\alpha$ - and  $\beta$ -blocking agents. Both phentolamine and propranolol are able to produce an almost instant effect on the vasoregulatory system. The effectiveness of  $\alpha$ - and  $\beta$ -receptor blockades was demonstrated by the absence of tachycardia and vasodilation when injecting nor-adrenaline (10 $\mu$ g, bolus), adrenaline (10 $\mu$ g, bolus) and isoprenaline (10 $\mu$ g, bolus) into the left ventricle.

#### **4.5.2 Discussion of Derived Parameters' Calculations**

##### **4.5.2.1 Calculation of Coronary Resistance**

Ohm's law for electricity defines resistance,  $R$ , as the the ratio of voltage drop,  $E$ , to current flow,  $I$ , so that  $E = I * R$ . Similarly, in fluid mechanics, the analogous hydraulic resistance or the resistance across a vascular bed,  $R$ , is usually calculated from the pressure drop across the bed divided by flow through it. Thus:

$$P_i - P_o = P_{\text{driving}} = R * Q$$

as illustrated in Figure 4.17

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<sup>1</sup>Refer to chapter 3.



Fig. 4.17: Diagrammatic representation of resistance calculation

Calculation of absolute coronary vascular resistance needs the effective downstream pressure representing the height of the "vascular waterfall" [Bellamy, 1978; Permitt, 1963] when the downstream pressure is higher than the external pressure of the vessels. This is not a measurable quantity in our experimental setup. Due to multiple factors involved in determining coronary resistance apart from vascular tone, perfusion pressure and blood viscosity, more complex influences arise from the heterogeneity of behaviour in different layers of the left ventricular wall and the alteration of the regional blood distributions. As Hoffman [1990] pointed out, it is not possible to calculate accurate coronary resistance ( $cr$ ) when you can not set up steady states for various parameters.

Even so, this work still attempts to calculate  $cr$  by using the formula proposed by Kilpatrick [1989] in our laboratory, as shown in equation 4.1. This formula is based on the assumption that there is a linear gradient of resistance across the myocardial wall and a linear distribution of coronary arterioles throughout the myocardial wall. As is well known [Berne, 1979], myocardial perfusion is mainly determined by perfusion pressure and  $cr$ . If we can calculate  $cr$  which is still believed to be determined by metabolic factors, we may be able to exclude the perfusion pressure effect on coronary circulation. Thus resistance can be shown diagrammatically in Figure 4.18. If we assume the material across left ventricular wall is uniform, the left ventricular pressure ( $LVP$ ) and coronary sinus pressure ( $csp$ ) both tend to collapse the coronary vessels, and the effect of these two pressures on the coronary resistance should be linear across the ventricular wall. In the centre of the ventricular wall, this pressure is equal to the mean of  $LVP$  and  $csp$ . During systole,  $LVP$  is much greater than  $csp$  and  $csp$  will have no effect on coronary resistance vessels due to the waterfall effect. Also during systole, distribution of coronary flow

across left ventricular wall is very uneven [Downey, 1974]. During diastole, however, the distribution of CBF has been shown to be usually very uniform across

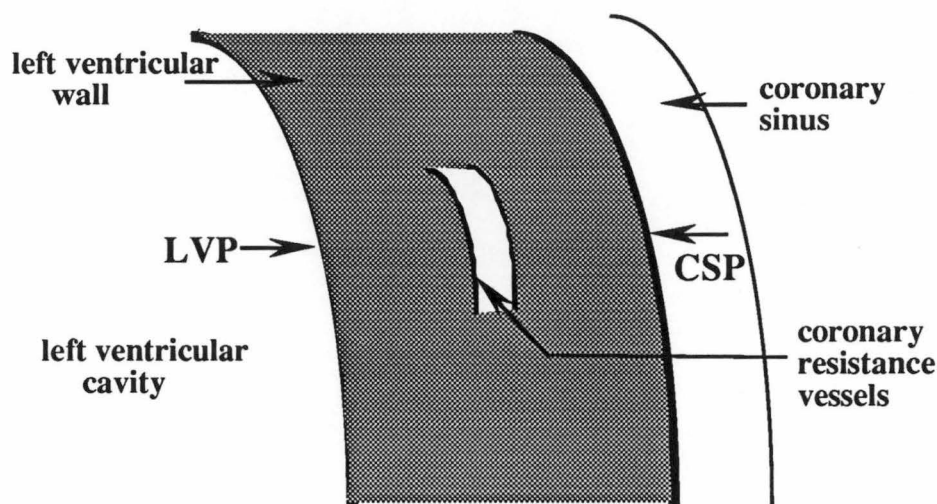


Fig. 4.18: Coronary resistance of the whole system

the ventricular wall in the normal beating heart in anaesthetized dogs [Becker, 1973; Buckberg, 1975; Downey, 1974; Hoffman, 1990]. Therefore, although the assumption that there is a linear distribution of coronary arterioles across myocardial wall is not strictly anatomically accurate, for diastolic coronary flow the errors may be assumed negligible. Thus formula 4.1 is reasonable for the estimation of  $cr$  during diastole.  $cr$  for this work was calculated by using formula 4.1 in programme *mindst* on each digitizing sample. The diastolic  $cr$  were averaged, beat by beat, to minimize the effects of systolic compression on the calculated coronary resistance [Denison, 1956].

#### 4.5.2.2 Calculation of Cardiac Work

The energy needed for cardiac work performance mainly comes from aerobic chemical metabolism, *ie* substrata are combined with oxygen to form water, carbon dioxide and energy [Elzinga, 1983; Berne, 1981]. The whole cardiac energy requirement can be divided into two parts, internal work and external work. The former may be defined as the work for metabolic processes which produces heat

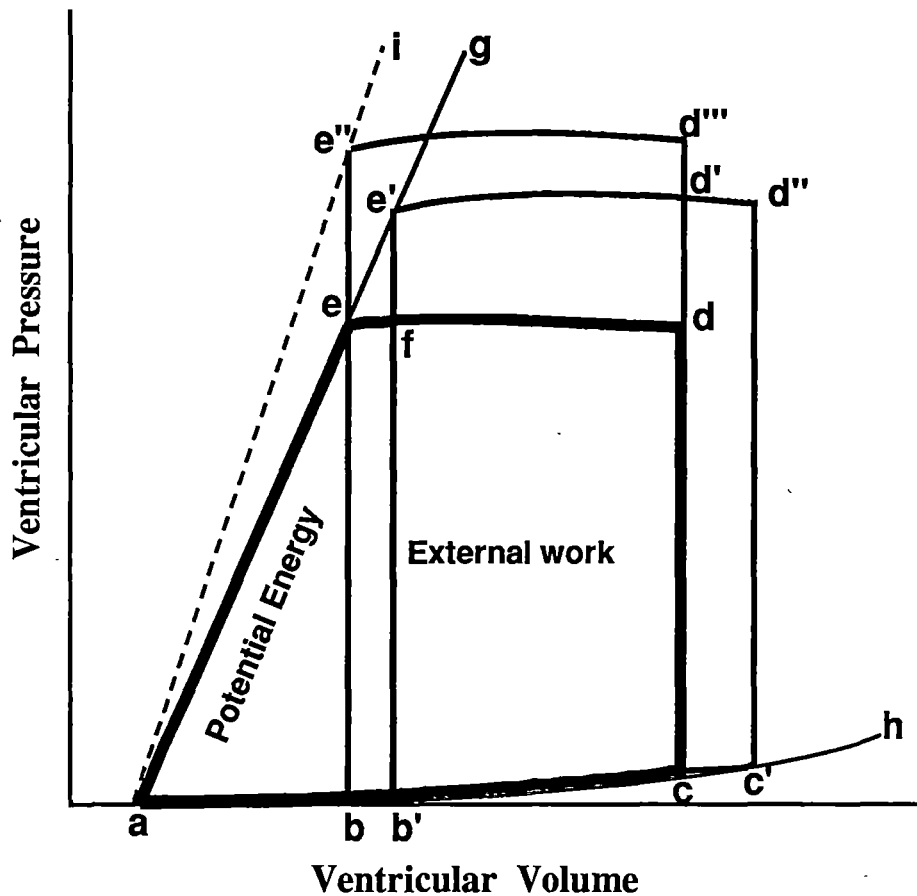
inside the heart and is difficult to calculate because both the substrata used by the heart to liberate energy, and the precise value of energy equivalent of the substrata burnt by the heart, remain unknown. In contrast, external work can be calculated without any significant assumptions about energy equivalent and substrata. This work is the energy needed for ejecting blood into the aorta from the left ventricle and we can directly measure the amount of blood ejected by the left ventricle and the pressure at which this ejection occurs. There are two components for the external work performed by left ventricle: *kinetic work* to accelerate the blood a certain velocity and *potential work* to restore the energy. In the normal beating heart, during diastole most of the energy is potential – storing energy for the ventricle ejection and during systole most of the energy is transformed from potential to kinetic. The kinetic work can be calculated from the equation [McDonald, 1974]:

$$\dot{w}_k = \frac{1}{2} \rho \frac{F^3}{A^2}$$

where  $\dot{w}_k$  is kinetic power,  $\rho$ , the density of blood,  $F$ , volume flow and  $A$ , the internal cross-sectional area of the aorta. The potential work can be calculated by equation 4.2 [Milnor, 1966].

Thus, if the cardiac output is constant during the intervention which we apply to the heart, it is reasonable to only use potential power (equation 4.2) to estimate the work done by the heart. This is the case in this investigation. An aortic band was used to increase cardiac work, which is pressure work. Even though the aortic band decreases the cross-sectional area of the aorta, the change is small. As I consider the amount of kinetic power out of the total cardiac work is negligible, equation 4.2 is reasonable for measuring cardiac work. Because the cardiac output is constant during increased cardiac work by aortic constrictions, it is reasonable to only use the proximal aortic pressure as an estimate of cardiac work.

The left ventricular pressure-volume area (PVA) concepts can further prove an obvious increase in cardiac work during increased proximal aortic pressure by using aortic band. During its cycle, the heart traces the loop **a-b-c-d-d-e-a** shown in Figure 4.19 – produced when instantaneous ventricular pressure is plotted against instantaneous ventricular volume.



**Fig.4.19:** Schematic illustration of pressure-volume area (PVA), containing two components-external mechanical work and potential energy. (modified from Suga [1979, 1980, 1982], see text for explanations)

The significance of this loop has been well-documented by Suga and Sagawa [Khalafbeigui, 1979; Suga, 1980; 1982]. **a-h** is the end diastolic pressure-volume relation curve and **a-g** is the end systolic pressure-volume line. The loop **b-c-d-e-b** represents four phases in the cardiac cycle:

*isovolumetric relaxation phase*  
*rapid filling phase*

**e-b line**  
**b-c line**

*isovolumetric contraction phase*  
*ejection phase*

**c-d line**  
**d-e curve**

As Suga *et al* point out, **PVA** in a normal pumping cycle consists of two areas: one is the rectangular area (loop **b-c-d-e-b**) which represents the external mechanical work and the other is the triangular area (loop **a-b-e-a**) and which represents the potential energy. The whole area represents the total mechanical energy generated by each cardiac cycle and has been used as the index of myocardial oxygen consumption. The product of flow and pressure (equation 4.2) integrate over a cycle produces the external stroke work which is the area in the loop **b-c-d-e-b**.

As the proximal aortic pressure was increased by the aortic band, the changes in the **PVA** shown in Figure 4.18 would occur:

- *Increasing left ventricular pressure without changing ventricular volume gives the loop **b'-c-d'-e'-b'**. The external work increase is dependent on the difference between the **e'-f-d-d'-e'** area and the **e-b-b'-f-e** area. Overall, the **PVA** is increased.*
- *Increasing left ventricular pressure associated with a decrease of left ventricular output, causes the ventricular volume to increase (**c** shifts to **c'**). In this situation, external work is the loop **b'-c'-d''-e'-b'**. Both external and internal work increase. **PVA** (loop **a-c'-d''-e'-a**) increases as well.*
- *Increasing the afterload of the left ventricle, the heart may increase its contractility in order to maintain the original cardiac output. This will induce a shift of **a-f** line to **a-i**, so that the loop **b-c-d'''-e''-b** represents the external mechanical work. Overall, the **PVA** (loop **a-c-d'''-e''-a**) increases.*

Overall, increasing the proximal aortic pressure does increase cardiac work, however, it may not always increase external work. Equation 4.2 may not accurately represent the working output of the heart. It can only be used as an estimate.

#### 4.5.2.3 Calculation of Myocardial Oxygen Consumption

The volume of oxygen consumed by the heart is determined by the cardiac activity and performance. **ATP** is the principle source of chemical energy in the heart under aerobic conditions.  $\dot{MVO}_2$  is closely coupled with the utilization of **ATP**. Even though the cardiac venous blood oxygen saturation is normally low compared to other systemic venous blood, the myocardium can still extract more oxygen from the coronary system under certain conditions.

Due to the difficulty of making direct measurements of  $\dot{MVO}_2$  in the closed-chest animal, 25 different indexes of  $\dot{MVO}_2$  have been proposed over the past 70 years based on the assumption that total mechanical work done by the ventricle correlates closely with  $\dot{MVO}_2$  [Baller, 1979]. The relationship between calculated  $\dot{MVO}_2$  index and exact  $\dot{MVO}_2$  index has been compared in the isolated heart with a high correlation being repeatedly observed from many hearts over a wide range of  $\dot{MVO}_2$  values [Baller, 1979, 1981; Suga, 1987; Rooke, 1982]. A considerable variation of interrelationship has also been shown amongst hearts [Burkhoff, 1987; Schipke, 1990; Suga, 1987]. This variation is attributed to such factors as biological variation, the difference of metabolic substrata utilization, and a varied proportion of **ATP** sources. The relationship between three well-accepted indices of  $\dot{MVO}_2$ , **PVA** [pressure-volume area, Suga, 1987], **PWI** [pressure-work index; Rooke, 1982] and **Et** [total energy requirement; Bretschneider, 1972], and the actual  $\dot{MVO}_2$  was found to alter with changes in afterload condition and contractile state [Schipke, 1990]. These authors concluded that each index had shortcomings in being able to predict  $\dot{MVO}_2$  accurately over a wide range of hemodynamic conditions.

Fortunately, the special hemiazygos vein system in the sheep enables us to measure **CS** oxygen content directly (see appendix C). Therefore, we simultaneously withdrew blood samples from the proximal aorta and the coronary sinus for measurements of oxygen saturation and haemoglobin as well as blood gases and pH.

Blood oxygen content was calculated using oxygen 1.34ml/g Hb when fully saturated plus dissolved oxygen 0.0031ml/mmHg pO<sub>2</sub> per 100mls of blood<sup>1</sup> as followed:

$$\text{O}_2 \text{ content} = 1.34 [\text{Hb}] \times \text{SO}_2(\%) \times 1/100 + 0.0031 \text{ pO}_2 \quad (4.9)$$

where [Hb] is haemoglobin concentration (g/dl) of the sample, SO<sub>2</sub> is oxygen saturation(%) and pO<sub>2</sub> is oxygen pressure in Torr. Both were measured by ABL3 Acid-Base Laboratory. Thus ventricular  $\dot{\text{MVO}}_2$  will be calculated from the product of CBF and arteriovenous oxygen content difference as equation (4.3). It is necessary to point out that the coronary sinus only drains most of the circumflex inflow. However equation 4.3 is reasonable for estimating  $\dot{\text{MVO}}_2$ .

#### 4.5.2.4 Significance of the Half Life of Response

It has been observed [Feigl, 1983] that a change in demand of the heart for oxygen will induce alteration of coronary circulation. The two interventions I applied to the sheep both cause a change of cardiac metabolic state, which elicits the vasoregulation of CBF. Let us assume that there is one substance X or a group of substances acting as one which is responsible for this vasoregulation. Then applying the intervention to sheep would cause the production and accumulation of this substance X. As the concentration of substance X increases in the interstitium, it induces vasodilation in the coronary circulation which causes the fall of coronary resistance in the arterial system. As soon as the intervention ceases and myocardial cell is back to normal condition, the concentration of substance X declines and the change of coronary vasodilation would recover to basal condition. This thesis primarily investigates the decay response of coronary circulation after interventions.

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<sup>1</sup>These figures were obtained on dogs. It may not be the same for the sheep as the sheep has two types of hemoglobin, whose structure can be quite different from that in dogs.



If a substance **X** is responsible for coronary vasoregulation during these interventions, then when the intervention stops **X** can either

- (a) *decay by itself - because it is inherently unstable; or*
- (b) *decay in a reaction with a scavenger.*

In case (a), we have true first order kinetics [Mayer, 1980]:

$$\frac{\partial[X]}{\partial t} = -k[X]$$

where  $[X]$  is the concentration of **X**. The solution is the familiar exponential decay:

$$[X] = [X]_0 e^{-kt}$$

where  $[X]_0$  is the concentration of **X** when the intervention ceases.

In case (b), we have a reaction:



where **S** is the scavenger and **X'** and **S'** are the products of the reaction of **X** and **S**.

We now have second order kinetics:

$$\frac{\partial[X]}{\partial t} = -k[X][S]$$

where  $[S]$  is the concentration of the scavenger. Since the concentration of **X** will be very low and the scavenger is likely to be present in much larger concentrations (**S** could even be water), it is very likely that

$$[S] \gg [X]$$

so that  $[S]$  will remain essentially constant during the decay of  $[X]$ . thus we can substitute a new constant:

$$k' = k [S]$$

and obtain *pseudo-first-order* kinetics.

In this thesis, I have reported  $k$  or  $k'$  as the half-life,  $t_{1/2}$  - the time for any concentration of  $X$  to decay to half its original value:

$$t_{1/2} = \frac{\ln 2}{k}.$$

In conventional kinetic studies, it is assumed that the active component,  $X$ , will eventually decay completely, *ie*

$$[X]_{\infty} = 0$$

However, if  $X$  is also the primary agent for basal vascular tone, its concentration cannot decay completely. In addition to the scavenging of  $X$  by  $S$ , there must be continuous production of  $X$  from some source, thus we have:

$$\frac{\partial [X]}{\partial t} = k'' - k' [X] \quad (4.10)$$

In the absence of the intervention, we have the equilibrium situation:

$$\frac{\partial [X]}{\partial t} = 0$$

and

$$k'' = k' [X]_e \quad (4.11)$$

Integrating (4.10):

$$\int_0^t \frac{\partial [X]}{k'' - k' [X]} = \int_0^t \partial t$$

Evaluating the integrals:  $\left| -\frac{1}{k'} \ln(k'' - k' [X]) \right|_0^t = t_0$

Expanding: 
$$\frac{1}{k'} \left( -\ln(k'' - k'[X]) + \ln(k'' - k'[X]_0) \right) = t$$

For convenience, put 
$$a = (k'' - k'[X]_0)$$

Rearranging: 
$$\ln\left(\frac{k'' - k'[X]}{a}\right) = -k' t$$

Taking the exponential of both sides:

$$\frac{k'' - k'[X]}{a} = e^{-k' t}$$

and rearranging: 
$$k'' - k'[X] = a e^{-k' t}$$

$$[X] = \frac{1}{k'} (k'' - a e^{-k' t})$$

Thus  $[X]$  will still show an exponential decay with the same decay constant,  $k'$ .

Thus, if substance  $X$  is a single chemical, which decays with first- or pseudo-first-order kinetics, and the response induced by increased afterload is caused by this single agent, the measured  $t_{1/2}$  should be constant regardless of the degree and duration of increased afterload.

Furthermore, if neural control and humoral control is also involved in this coronary vasoregulation, four possibilities could occur after using blockers if the blocking effect is complete:

- *Both the vasodilating response and  $t_{1/2}$  after different interventions could remain the same, suggesting that the vasoregulation is caused by a single mechanism and is not related to neural and humoral control.*
- *The response remains the same, but  $t_{1/2}$  changes to another relatively stable value. This means that there is direct neural and/or humoral control of*

*production of substance X. However, the body can now compensate through other chemicals when that basic route is blocked in order to keep blood supply to the coronary circulation.*

- *The  $t_{1/2}$  stays the same, but the response changes. This can be explained if neural and humoral controls are only indirectly involved in the release of substance X. The blockers of these agents only change the concentration of substance X.*
- *Both the  $t_{1/2}$  and response are changed relatively to a new stable value. In this case, humoral and neural controls are likely to be directly involved in the normal vasoregulation under those interventions.*

Thus, if complete blocking agents can be selected for sympathetic, parasympathetic and humoral (adenosine and **EDRF**) control, it is possible to search for the mechanism for the vasoregulation under these interventions from the changes of the response and half life. In addition, it is possible to identify substance X by testing the half lives of those chemicals proposed to be responsible for coronary vasoregulation one by one.

## **CHAPTER 5**

### **RESULT 1 – DECAY OF CORONARY VASODILATION IN RESPONSE TO INCREASED CARDIAC WORK**

## **5.1 RESULTS**

### **5.1.1 Hemodynamic Assessment of Increased Cardiac Work**

Table 5.1 gives the ranges of heart rate, systolic and diastolic aortic pressure, left ventricular diastolic pressure, mean pulmonary blood flow, blood acid-base balance and electrolyte analysis on samples withdrawn from both proximal aorta and coronary sinus in response to 25-44% increase in proximal systolic aortic pressure for a 120 second period. Because the experiments were performed in the open-chest whole sheep, baseline hemodynamic variables differed from sheep to sheep. There was no significant difference between heart rates, diastolic aortic pressure and diastolic left ventricular pressure. A statistical difference between control and stimulus was found in the pH of proximal aortic blood, but not in the coronary sinus blood. Significant differences in  $p\text{CO}_2$  were found in the coronary sinus, but not in the artery. The range of differences were small. The changes of  $p\text{O}_2$  and  $\text{SO}_2$  were much more complicated than Table 5.1 shows. These differences would be discussed in chapter 7. In the control group, I have randomly selected three sheep to study the sequence of response throughout a whole experiment lasting 3-4hours. The sheep showed similar magnitude responses as time passed. This suggests that the responses of sheep to interventions were not related to the sequence of interventions applied.

In summary, the stimulus applied for this study did not produce significant changes in heart rate, diastolic aortic pressure and cardiac output. Thus, in this chapter, I use the changed systolic pressure to express the change of cardiac work<sup>1</sup>.

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<sup>1</sup>Refer to section 4.5.1.2.

**Table 5.1: Hemodynamic Variables and Indexes of Myocardial Metabolism during increased cardiac work**

			Aortic Constriction (changes of range)			
Control						
			on	P value	off	P value
Heart rate (beats/min) (DF=12)		104.6±2.6	103.24±2.6	n.s.	104.0±3.1	n.s.
Systolic aortic pressure		rely on different degree of constriction				
Diastolic aortic pressure (DF=11)		80.7±3.0	79.9±3.5	n.s.	79.7±4.07	n.s.
Diastolic L.V. pressure (DF=11)		10.5±0.8	11.0±0.7	n.s.	10.0±0.9	n.s.
pqm (DF=11)		0.9±0.05	0.9±0.07	n.s.	0.9±0.06	n.s.
pH (DF=21)	Aorta	7.48±0.02	7.49±0.02	<0.05	7.47±0.02	n.s.
	C.S.	7.41±0.02	7.42±0.02	n.s.	7.42±0.02	n.s.
pCO2 (DF=21)	Aorta	32.5±1.3	32.5±1.6	n.s.	32.8±1.5	n.s.
	C.S.	38.6±1.4	39.4±1.6	<0.05	40.0±1.7	n.s.
pO2 (DF=21)	Aorta	102.11±6.97	111.4±7.0	<0.05	115.4±16.3	n.s.
	C.S.	26.2±1.09	27.5±1.07	n.s.	24.5±0.95	<0.05
HCO3 (DF=21)	Aorta	30.5±0.74	31.1±0.87	<0.01	30.8±0.85	n.s.
	C.S.	32.2±0.58	33.7±0.75	<0.01	34.1±0.8	n.s.
TCO2 (DF=21)	Aorta	31.1±0.79	31.7±0.95	<0.05	31.5±0.88	n.s.
	C.S.	33.1±0.63	34.6±0.78	<0.01	35.0±0.84	n.s.
SO2 (DF=21)	Aorta	95.8±1.07	96.9±1.03	<0.05	96.78±0.96	n.s.
	C.S.	58.0±3.03	59.9±3.16	n.s.	54.8±3.14	<0.05
K+ (n=3)	Aorta	2.5	2.55		2.6	
	C.S.	2.7	2.5		2.5	
Na+ (n=3)	Aorta	139	136		141	
	C.S.	144	143		140	

control - control data before aortic constriction; on - data at 2minutes after aortic constriction  
off - data at 30 seconds after aortic constriction;

P value - two tailed probability of difference of paired data between control to on and on to off:

P<0.05 - statistically significant difference; n.s. - not statistically significant;

DF- degree of freedom (n-1);

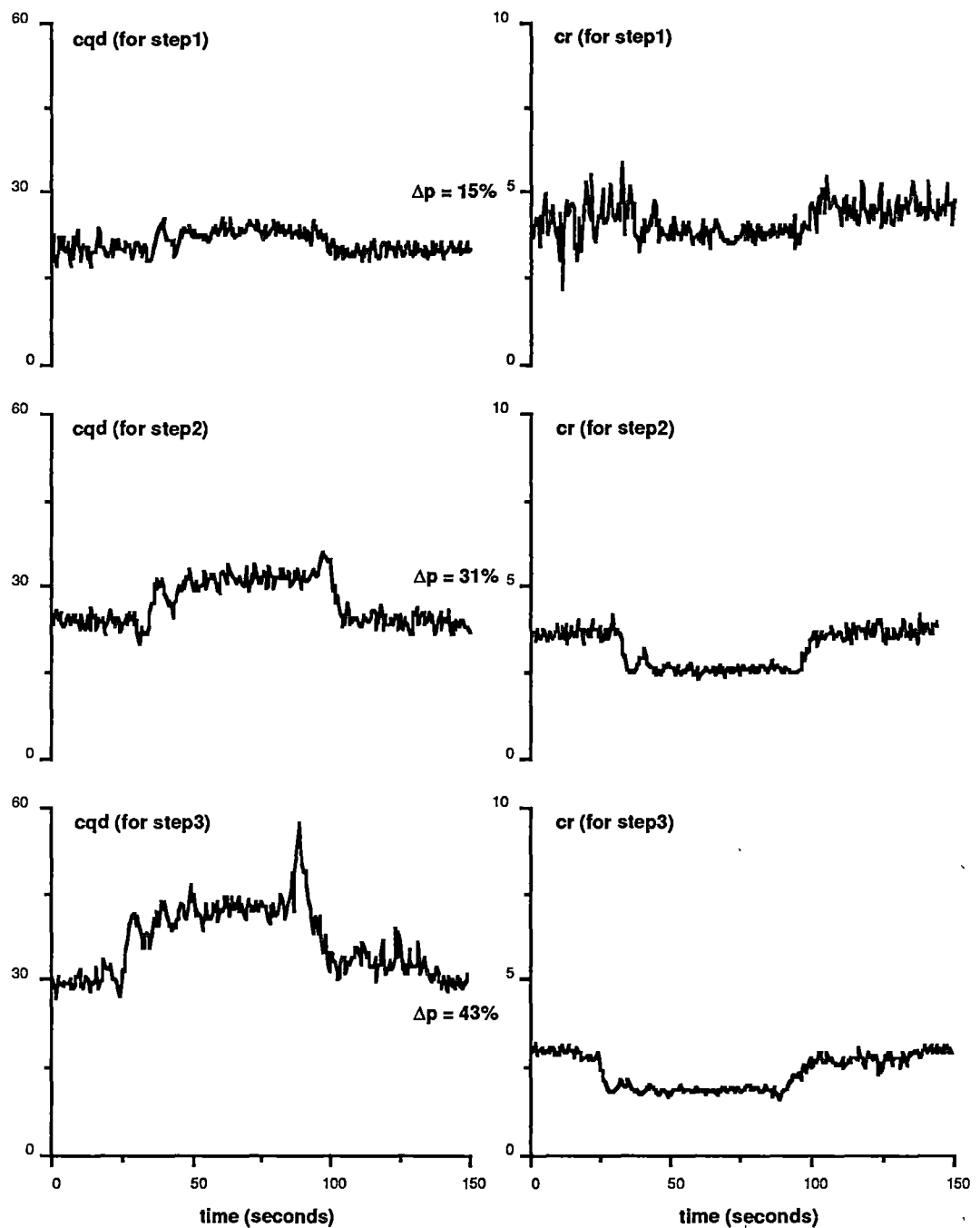
Aorta - sample from proximal aorta; CS - sample from coronary sinus;

All data are expressed as MEAN±SEM.

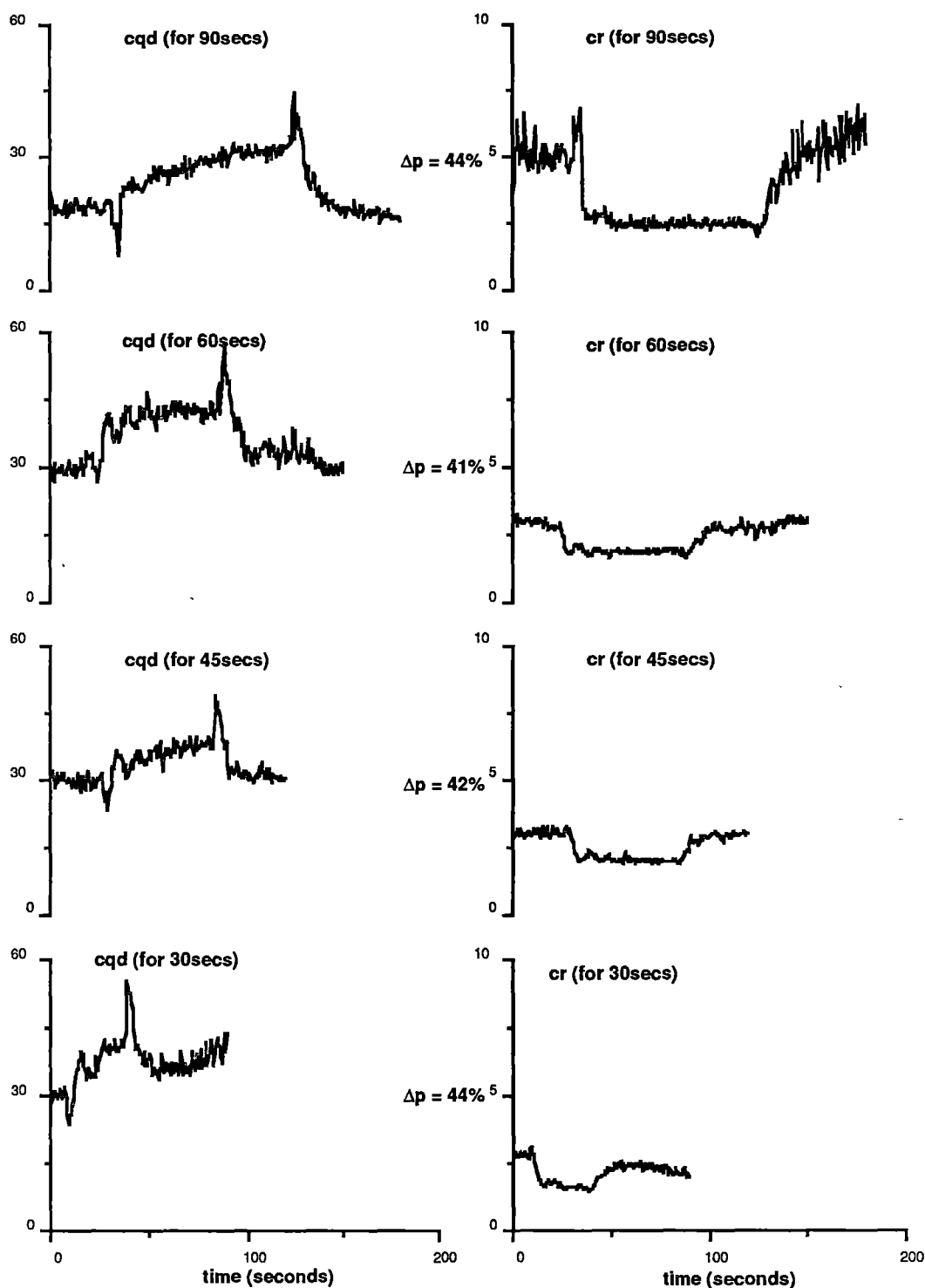
### **5.1.2 Half-Life and Degree and Duration of Increased Cardiac Work Before any Antagonists**

Figures 5.1 and 5.2 show the changes of coronary resistance and mean diastolic coronary blood flow in response to different degrees of increased cardiac work in one experiment (5.1) and to the different periods of increased cardiac work (5.2). Figure 5.1 demonstrated that the more severe the increased aortic pressure is, the more **CBF** and **cr** change. Their good correlation from a single sheep experiment was shown in Figure 5.3. However, the **CBF** and **cr** did not change further following the longer period of the increased aortic pressure (Figure 5.2).

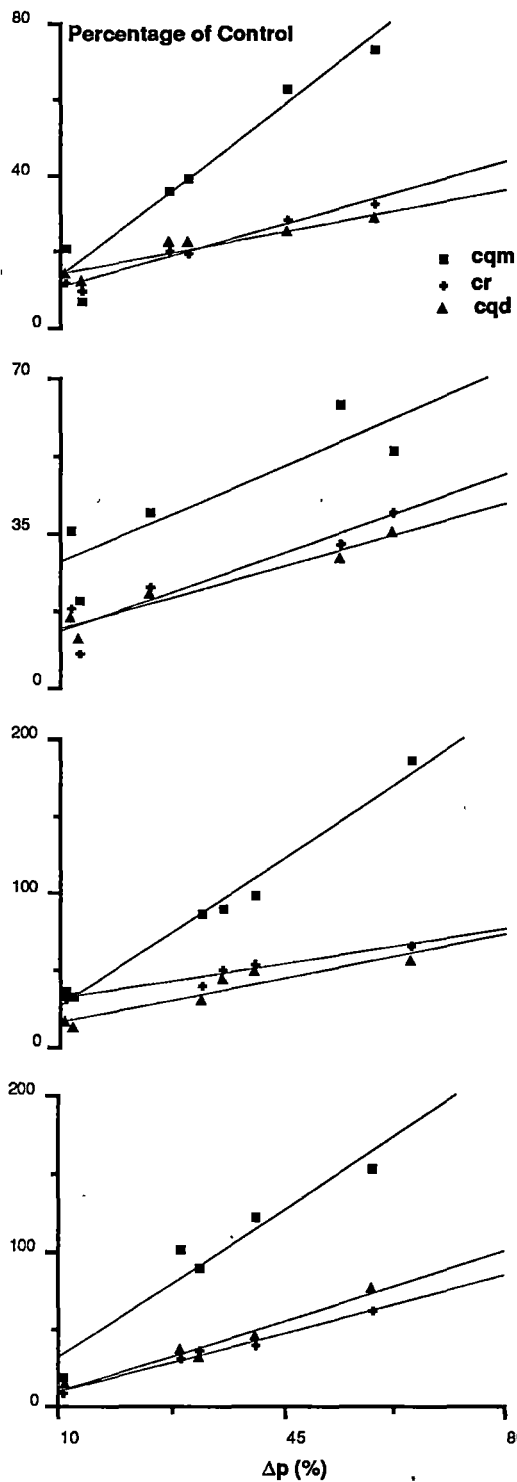




**Fig.5.1:** Mean diastolic coronary blood flow and coronary resistance in response to different degrees of aortic constriction with the same duration (60secs). ( $\Delta p$  - percentage change of systolic aortic pressure; **cqd** - diastolic coronary blood flow; **cr** - diastolic coronary resistance).



**Fig.5.2:** Mean diastolic coronary blood flow and coronary resistance in response to 90, 60, 45 and 30 seconds of aortic constriction with same degree. ( $\Delta p$  - percentage change of systolic aortic pressure; **cqd** - diastolic coronary blood flow; **cr** - diastolic coronary resistance).



**A: Duration = 30 secs**

The equations of the levels of best fit are:

$$\Delta cq_m = -5.12 + 1.82 \Delta p \quad R = 0.949$$

$$\Delta cr = 3.98 + 0.66 \Delta p \quad R = 0.955$$

$$\Delta d.cq = 8.91 + 0.46 \Delta p \quad R = 0.902$$

**B: Duration = 45 secs**

The equation of the levels of best fit are:

$$\Delta cq_m = 19.37 + 0.88 \Delta p \quad R = 0.759$$

$$\Delta cr = 6.01 + 0.60 \Delta p \quad R = 0.876$$

$$\Delta d.cq = 8.01 + 0.56 \Delta p \quad R = 0.906$$

**C: Duration = 60 secs**

The equation of the levels of best fit are:

$$\Delta cq_m = -1.85 + 2.77 \Delta p \quad R = 0.982$$

$$\Delta cr = 24.46 + 0.65 \Delta p \quad R = 0.94$$

$$\Delta d.cq = 7.40 + 0.81 \Delta p \quad R = 0.875$$

**D: Duration = 90 secs**

The equation of the levels of best fit are:

$$\Delta cq_m = 4.18 + 2.72 \Delta p \quad R = 0.928$$

$$\Delta cr = -1.35 + 1.08 \Delta p \quad R = 0.986$$

$$\Delta d.cq = -4.21 + 1.31 \Delta p \quad R = 0.968$$

**Fig. 5.3:** Relationship between changes of  $cq_m$ ,  $cr$  and  $cq_d$  and increased proximal systolic aortic pressure for various durations (30, 45, 60, 90 secs) from one sheep ( $\Delta p$  - percentage of control in proximal aortic systolic pressure;  $cr$  - diastolic mean calculated coronary resistance;  $cq_m$  - mean coronary blood flow;  $cq_d$  - diastolic mean coronary blood flow).

The relationship between the calculated decay (half life) and the degree and period of change of percentage of systolic aortic pressure, which indicated the increased cardiac work, is shown in Figure 5.4. The half life is independent of the duration and degree of increased cardiac work. Figure 5.5 shows plots of the half life of the recovery of mean coronary blood flow, calculated diastolic mean coronary resistance and mean diastolic coronary blood flow *versus* percentage increase in aortic pressure. Half lives of **cqm**, **cr** and **cqd** were below 7seconds despite the large variation in aortic pressure. Similarly, the decay of three parameters (**cqm**, **cr**, **cqd**) *versus* the duration of increased aortic pressure was plotted in Figure 5.6. The values of these half lives were also below 7seconds. Finally, Figure 5.7 summarizes the average half lives in 12 sheep<sup>1</sup> in response to both different degree and duration of aortic constriction. Values are expressed as **mean±SEM** (seconds). The decay of the responses from 12 sheep are:

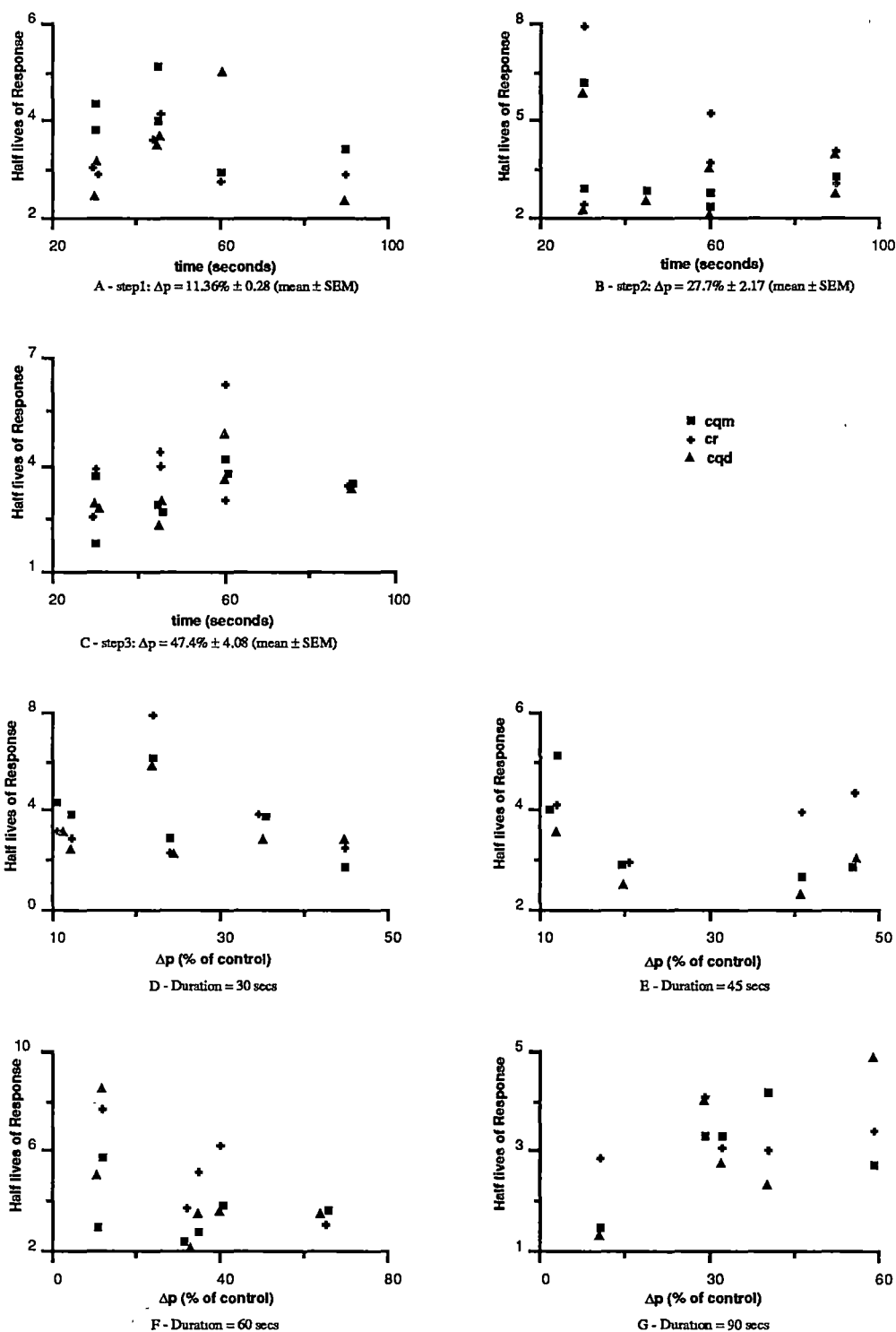
$$t_{\frac{1}{2}}(\mathbf{cqm}) = 4.67 \pm 0.17 \text{ (seconds)}$$

$$t_{\frac{1}{2}}(\mathbf{cr}) = 4.73 \pm 0.20 \text{ (seconds)}$$

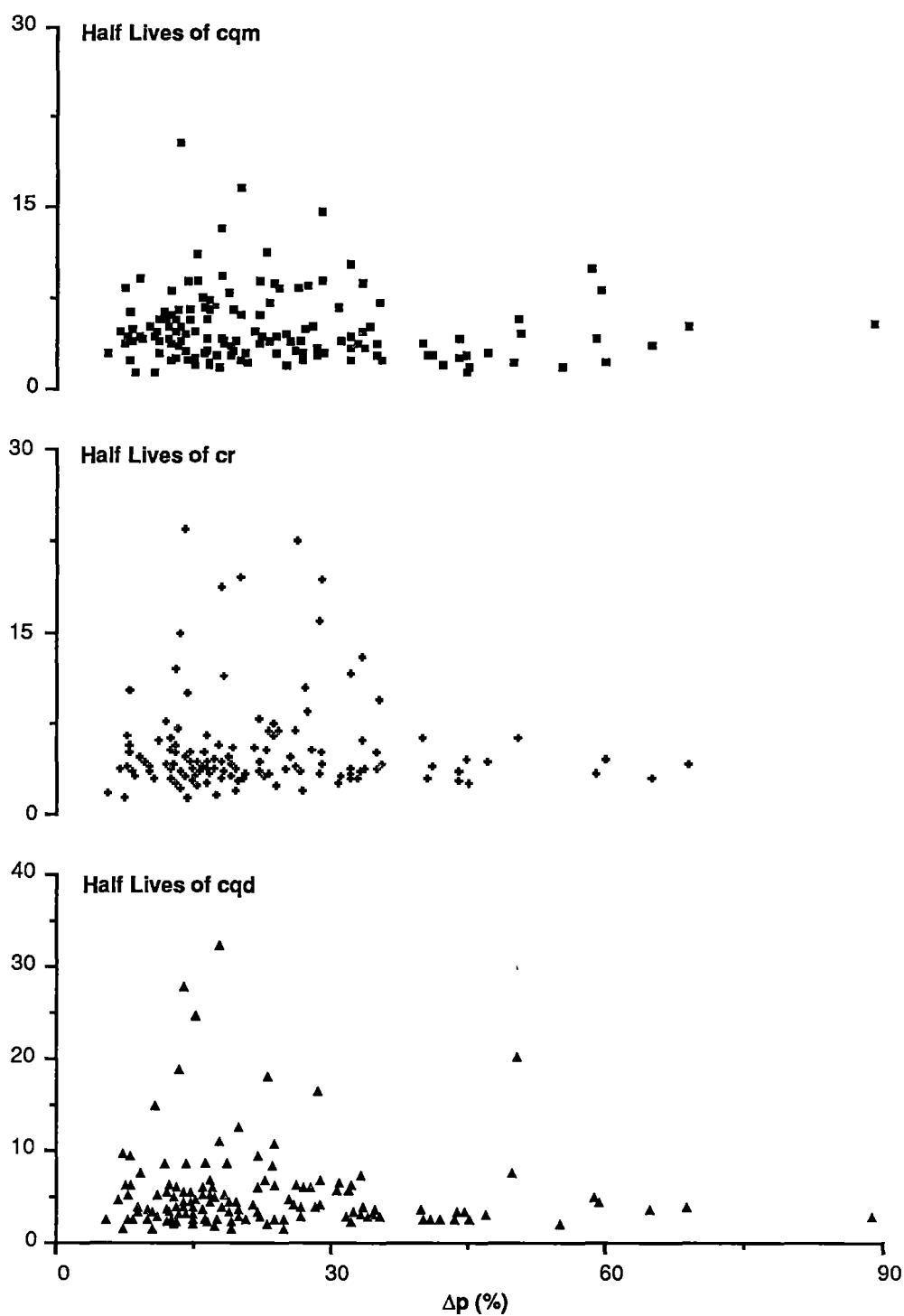
$$t_{\frac{1}{2}}(\mathbf{d.cq}) = 4.63 \pm 0.26 \text{ (seconds)}$$

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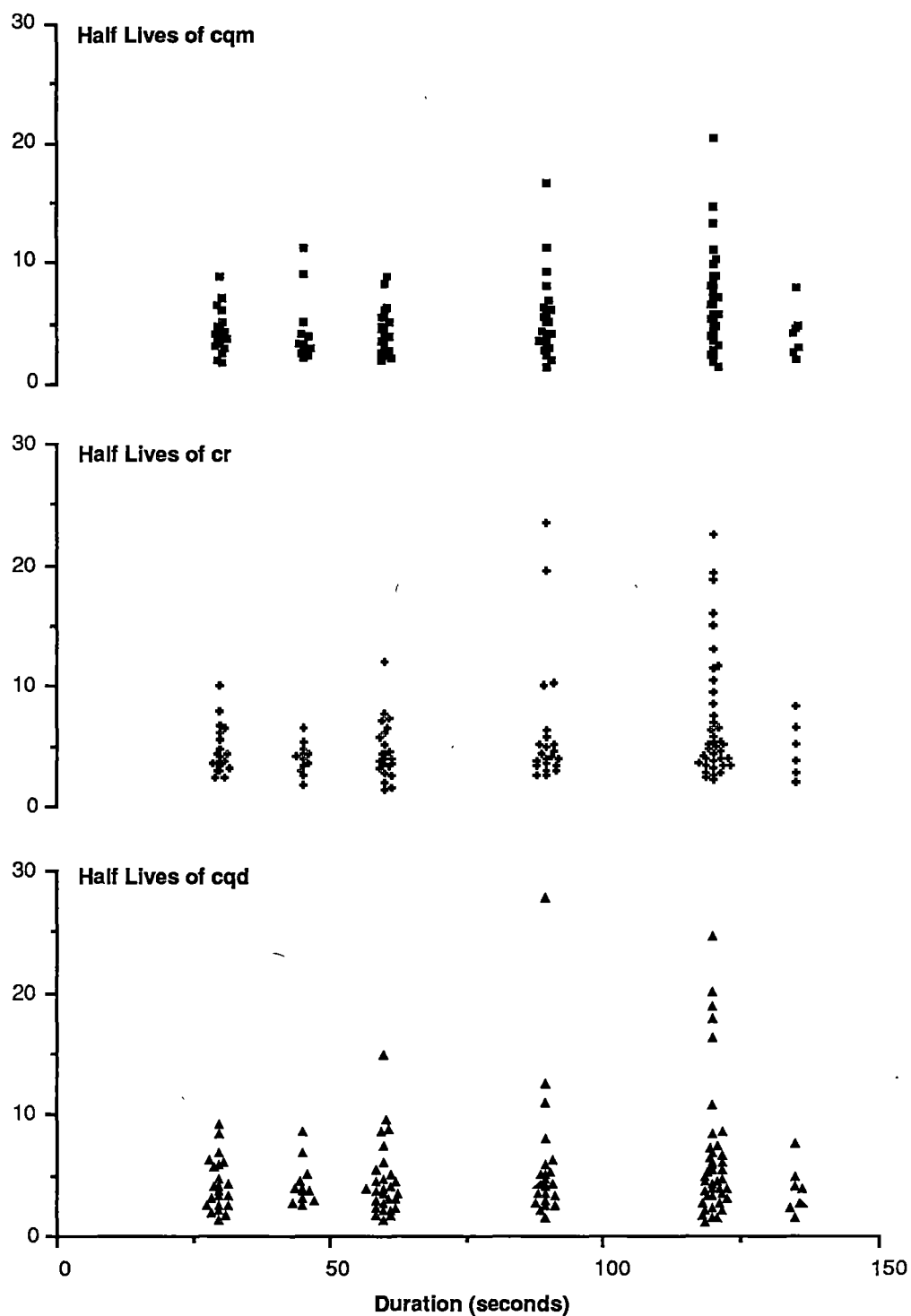
<sup>1</sup>One sheep with only 4 values was deleted from the results, leaving results from 12 sheep.



**Fig. 5.4:** Relationship between decay of cqm, cr and cqd and different degrees and durations of increased proximal aortic pressure from one sheep experiment. ( $\Delta p$  - percentage of control in proximal aortic systolic pressure; cqm - mean coronary blood flow; cr - diastolic mean calculated coronary resistance; cqd - diastolic mean coronary blood flow). See text for explanation.



**Fig. 5.5:** Decay of **cqm** (mean coronary blood flow), **cr** (diastolic mean calculated coronary resistance) and **cqd** (mean diastolic coronary blood flow) vs  $\Delta p$  (percentage of control in proximal aortic pressure). (Data were from 13 sheep with 143 points.)



**Fig. 5.6:** Decay of **cqm** (mean coronary blood flow), **cr** (mean diastolic calculated coronary resistance) and **cqd** (mean diastolic coronary blood flow) vs the durations of proximal aortic constriction. (175 data points from 13 sheep.)

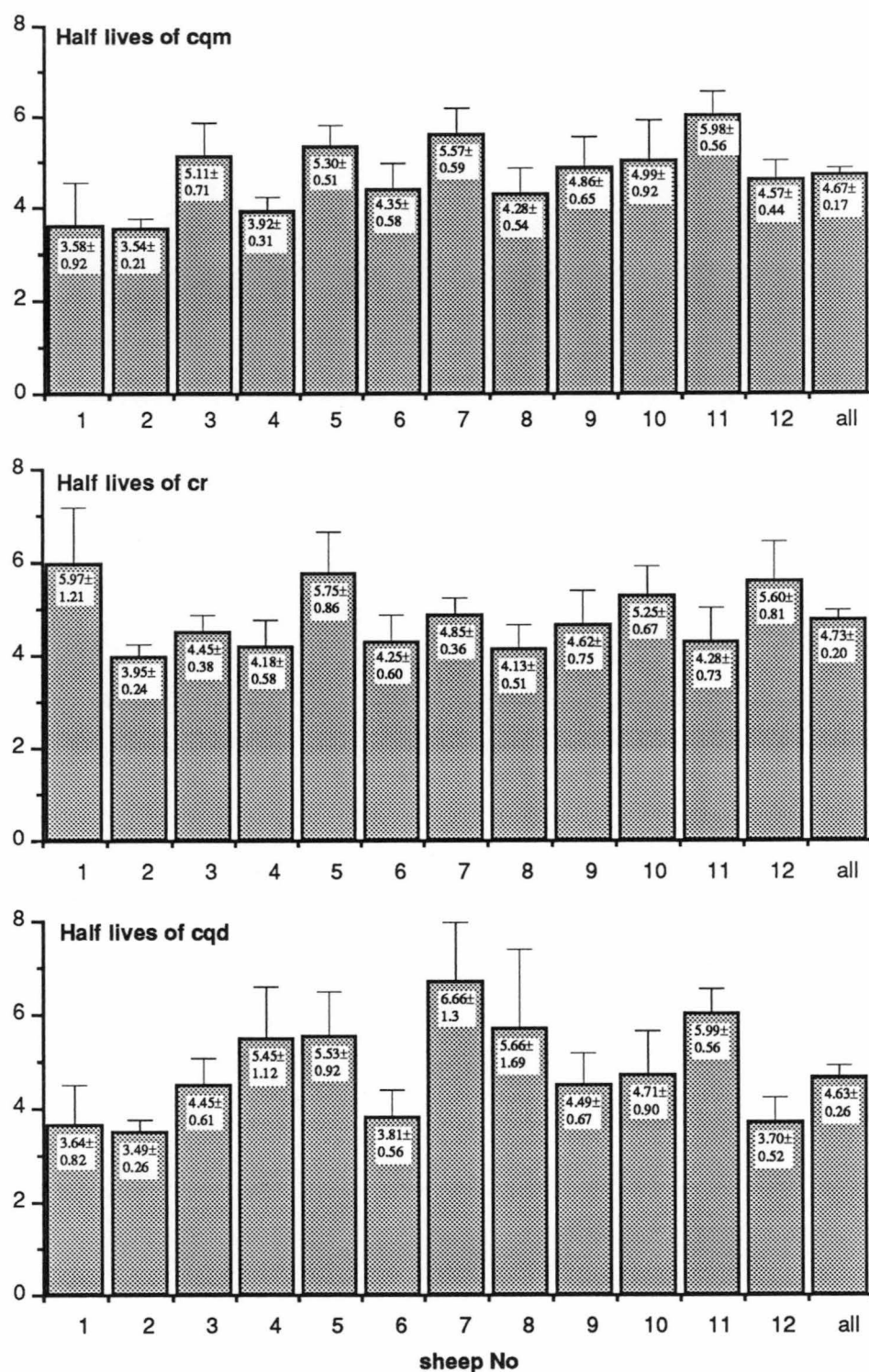


Fig. 5.7: Decay of **cqm** (mean coronary blood flow), **cr** (mean diastolic calculated coronary resistance) and **cqf** (mean diastolic coronary blood flow) in response to different degree and duration of aortic constriction in 12 sheep. Values are **mean ± SEM**. 'all' summarizes the data from 12 sheep.



### 5.1.3 Half-Life and Increased Cardiac Work After Using Adenosine, $\alpha$ and $\beta$ Receptor Inhibitions

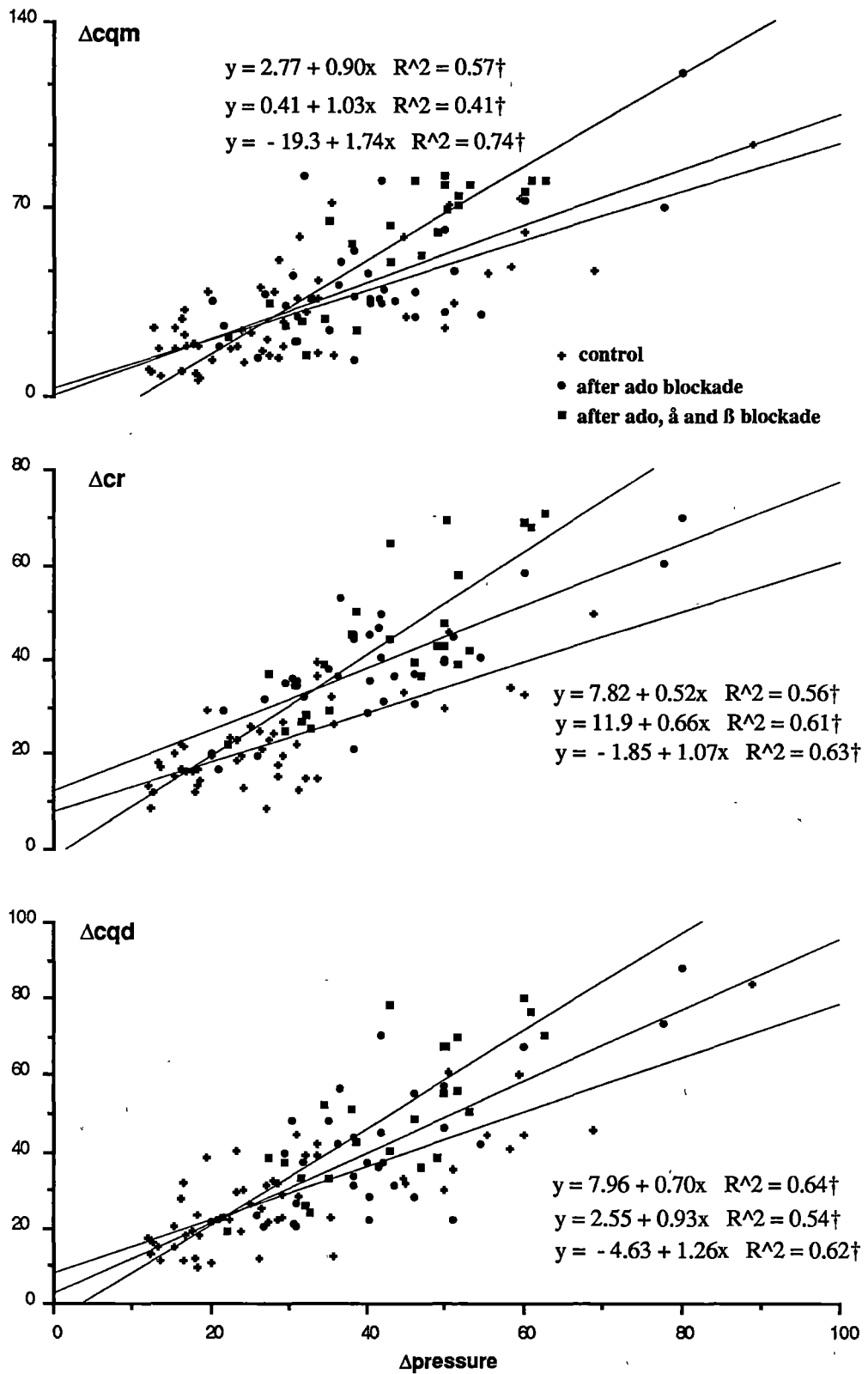
Each sheep in this group was subjected to various degrees of aortic constriction for a single duration (120 seconds). The relationships between the changes of **cqm**, **cr** and **cqd** and percentage change from control in proximal aortic pressure are shown in Figure 5.8 for each experimental state (control, adenosine blockade and adenosine,  $\alpha$ -,  $\beta$ -receptors blockade). There was a slight increase of slope in the relationship between response and pressure after using **8-PT** and more increase after using phentolamine and propranolol. The slopes are shown in Table 5.2<sup>1</sup>.

**Table 5.2:** Slopes of the relationship between coronary blood flow and coronary resistance before and after inhibitions

	Control & ADO blockade			Control & ADO, $\alpha$ and $\beta$ blockade			ADO blockade & ADO, $\alpha$ and $\beta$ blockades		
	$\Delta cqm$	$\Delta cr$	$\Delta cqd$	$\Delta cqm$	$\Delta cr$	$\Delta cqd$	$\Delta cqm$	$\Delta cr$	$\Delta cqd$
Slope 1	0.90	0.52	0.70	0.90	0.52	0.70	1.03	0.66	0.92
Slope 2	1.03	0.66	0.93	1.73	1.07	1.26	1.73	1.07	1.26
T value	-0.572	-0.758	-1.456	-3.212	-3.189	-2.835	-2.024	-2.231	-1.267
DF (n1+n2-4)	83	83	83	73	73	73	54	54	54
Student's t	1.990	1.990	1.990	2.648	2.648	2.648	2.009	2.009	2.009
P value	0.05	0.05	0.05	0.01	0.01	0.01	0.05	0.05	0.05
Conclusion	n.s.	n.s.	n.s.	s	s	s	s	s	s

Control - before inhibition; ADO - adenosine; n.s. - no significant difference; s - significant difference.

<sup>1</sup>Refer to section 4.3.4 for statistical method.



**Fig. 5.8:** Relationship between the change of cqm, cr and cqd and the percentage changes of proximal systolic aortic pressure due to aortic constriction for a duration of 120 seconds before and after adenosine,  $\alpha$ - and  $\beta$ -receptors inhibition. (Data are expressed as percentage of control from 7 sheep, each sheep included three types of experiment - control; adenosine blockade; and adenosine + adrenergic receptors blockade;  $^\dagger$ -significant association  $p < 0.01$ ).

Table 5.3 summarizes the results of experiments measuring the half-lives in response to increased cardiac work before and after using **8-PT**, phentolamine and propranolol in seven sheep.

**Table 5.3:** Mean half lives of decay before and after adenosine,  $\alpha$  and  $\beta$ -receptors blockade

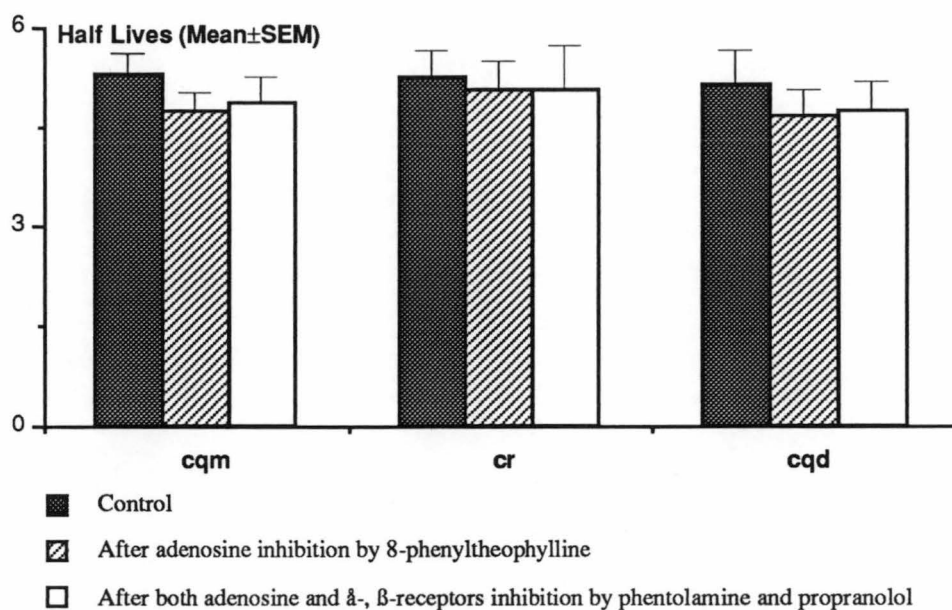
Sheep	Control			After ADO Inhibition			After ADO, $\alpha$ and $\beta$ blockades		
	cqm	cr	cqd	cqm	cr	cqd	cqm	cr	cqd
1	7.90	5.36	5.93	7.11	5.57	7.69	—	—	—
2	4.69	5.39	4.42	3.11	6.12	3.47	4.83	4.32	4.93
3	4.28	4.13	5.66	4.80	3.35	4.76	5.90	5.24	4.10
4	4.86	4.62	4.49	4.40	4.78	4.65	7.21	3.99	3.86
5	5.50	5.49	4.71	5.65	4.14	4.36	4.22	5.97	5.01
6	5.05	3.99	4.31	4.68	6.32	5.24	3.26	3.99	5.70
7	6.13	4.56	4.30	7.00	3.86	4.70	—	—	—
Mean	5.29	4.75	4.85	5.25	5.04	5.06	5.14	4.66	4.75
SEM	0.33	0.25	0.41	0.39	0.46	0.67	0.50	0.39	0.41

**cqm** - mean coronary resistance; **cr** - mean diastolic calculated coronary resistance; **cqd** - mean diastolic coronary blood flow; **ADO** - adenosine

Under control conditions, the decays of response following the withdrawal of various degrees of increased cardiac work for 120 seconds were:

**cqm**      **5.3±0.3** seconds;  
**cr**        **4.7±0.2** seconds;  
**cqd**      **4.8±0.4** seconds.

Neither **8-PT** nor phentolamine nor propranolol alter the decay of recovery in response to the withdrawal of stimulus as shown in Figure 5.9.



**Fig 5.9:** Mean decay (seconds) of **cqm**, **cr** and **cqd** before and after adenosine,  $\alpha$ - and  $\beta$ -receptor inhibitions. No significantly different from control was showed by paired  $t$ -test for both adenosine inhibition and adenosine,  $\alpha$ -,  $\beta$ -receptor inhibitions.

## 5.2 DISCUSSION

As the proximal aorta constricts, consequently increasing the afterload of left ventricle, the myocardial cells suddenly need to do more work to overcome the resistance. This increase of cardiac activity requires more energy which mainly comes from the blood supply. The way to meet this requirement is either to extract more nutrients from each ml of blood in the coronary circulation or to increase coronary blood flow. Since the coronary reserve is relatively small compared with that of other circulations, the coronary blood flow usually increases. The mechanism for this has not been ascertained.

The present results demonstrate that the degree of aortic stenosis is directly proportional to the cardiac work as long as the myocardium is not depleted<sup>1</sup> due to "over-load". So, the more severe the aortic constriction, the stronger cardiac activity is. The increased cardiac work will increase production of metabolite(s). Similarly, the longer the period of aortic constriction, the more sustained situation myocytes are in before the myocytes are exhausted. If this is true, it should cause more accumulation of metabolite(s), consequently a gradual increase in coronary blood flow and decrease in coronary resistance should be seen. In fact, I did not observe this: once the coronary resistance was stabilized after intervention, it tended to stay stable until the stimulus was withdrawn. If the metabolite(s) produced are responsible for the decrease of coronary resistance or increase of coronary blood flow and is substance X, when the stimulus stops, X can either decay by itself or decay in a reaction with a scavenger. Figure 5.5 and 5.6 shows the distribution of half lives against either degree or duration of stenosis. The degree and the duration does not affect the decay of the response as shown in figure 5.4. There is no correlation

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<sup>1</sup>Myocardial depletion will affect the myocardial constriction and may be shown as an increase in left ventricular end diastolic pressure. This was not observed in this study.

between the decay of response, in coronary blood flow, diastolic coronary blood flow and coronary resistance, and either the degree or the duration of the aortic constriction stimulus. In addition, a longer period of the intervention does not cause further change in coronary resistance. These results suggest that substance X is a single chemical with a short half life.

Despite the substantial evidence that adenosine is not responsible for the maintenance of coronary basal vasomotor tone, adenosine is still supposed by many laboratories to play an important role in coronary vasodilation in response to increased cardiac activity under the normal physiological conditions<sup>1</sup>. The present investigation was undertaken to determine whether the antagonism of adenosine on the coronary resistance vessels would impair the coronary vasodilation which occurs during increased cardiac work. The use of adenosine blockade changed augmentation of the responses shown by the slope in the relationship between coronary resistance and coronary blood flow and increased cardiac work, but this slight increase of slope was not statistically significant (Table 5.2). Thus, adenosine receptor blockade with 8-phenyltheophylline did not interfere with the relationship between the coronary resistance and cardiac work. This result is consistent with recent findings by Bache and co-workers[1988], who found that neither adenosine deaminase nor 8-phenyltheophylline changed the increase in coronary blood flow or the decrease in coronary venous oxygen tension that occurred during treadmill exercise in conscious dogs. Before concluding whether adenosine is a mediating factor for vasodilation during increased cardiac work, two points need to be considered:

- a) *Since 8-PT is a competitive adenosine receptor blocker and there is a very rich resource for adenosine production in the heart, it is possible for the system to generate more adenosine to compete the receptors with 8-PT. In that case, it seems rational to expect the decay of the response after the*

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<sup>1</sup>Refer to section 3.3.3.

*withdrawal of aortic constriction to stay the same after using an adenosine blocker and close to the decay of the response due to exogenous adenosine administration, assuming that extravascularly released adenosine and intravascularly administered adenosine have similar physiological effects. The half life of exogenous adenosine will be discussed in chapter 8.*

- b) Adenosine is a mediator. When the system is subject to adenosine blockade, it may be able to trigger another mechanism or release another metabolite to provide compensating response. If this were the case, the change of decay from the control should be observed after adenosine inhibition, and the half life of control should be very close to the decay of exogenous adenosine. No such alteration was found.*

These two possibilities would keep the relationship between the coronary resistance and cardiac work constant after adenosine inhibition. However, the observation that no significant difference was found between the half lives before and after adenosine inhibition (Table 5.3) suggests that substance X is a single metabolite, unless the compensatory metabolite after blockade has a similar half life to the control one. It is very likely the same metabolite was responsible for the reaction of both control and adenosine blockade groups. The question of whether it is adenosine will be examined by looking at the half life of exogenous adenosine.

For a better understanding of the response to increased cardiac work, it is important to clarify the involvement of sympathetic and parasympathetic systems in the changes of CBF and coronary vascular resistance. It has been well documented that the coronary vasculature is innervated by both the sympathetic and parasympathetic division of the autonomic nervous system [Berne, 1979; Feigl, 1983]. The data in Figure 5.8 and Table 5.2 show a significant increase of slope in the relationship between the change of reaction in  $c_{qm}$ ,  $c_r$  and  $c_{qd}$  and the change of aortic pressure after the sheep were blocked with 8-PT, phentolamine and propranolol. This indicates that the coronary circulation would dilate more in response to the same degree of cardiac work with  $\alpha$ - and  $\beta$ -adrenergic blockade than without them. This

suggests that coronary  $\alpha$ -receptor vasoconstriction must be dominant in coronary vasoregulation due to increased cardiac work caused by increase of the afterload on the left ventricle. When sheep were under complete blockade of  $\alpha$ - and  $\beta$ -receptors, there was no significant change of half lives from control. This suggests that the release of substance X is not controlled by neurogenic stimulation.

As the proximal aortic constrictions were performed, carotid sinus hypotension might occur. It was found that carotid sinus hypotension results in no change [DiSalvo, 1971] or decrease [Mohrman, 1978; Powell, 1979] in coronary resistance. With a beta-blocker (propranolol: 1mg/kg) and bilateral vagotomy, carotid sinus hypotension resulted in sympathetic coronary vasoconstriction in closed-chest dogs with controlled aortic pressure and myocardial oxygen metabolism [Powell, 1979]. This indicated that an  $\alpha$ -receptor coronary vasoconstriction from the carotid sinus reflex is independent of aortic perfusion pressure, myocardial metabolism and autoregulation. Another possibility is that the reduction of carotid sinus pressure could reach 50-60mmHg when aortic stenosis was applied with  $\alpha$  and  $\beta$  blockers. It was observed that a carotid sinus pressure below 50-60mmHg caused an activation of chemoreceptor fibres which produces reflex vagal parasympathetic coronary vasodilation [Vatner, 1975] in conscious dogs. This was found to be due to pulmonary hyperinflation when chemoreceptors were stimulated with nicotine. This would not be the case in anaesthetised animals with controlled ventilation. The use of propranolol in this study prevented an autonomic change in heart rate and contractility. This is not perfect prevention, further work should be carried out by using atropine and propranolol together to prevent parasympathetic effects.

In summary, the use of adenosine and  $\alpha$ ,  $\beta$  receptor blockers did not significantly interfere with the decay of the response and only changed augmentation of the vasodilation. This further supports the proposal that substance X is a single metabolite.



### 5.3 CONCLUSION

The recovery of coronary resistance to normal after the change of the afterload of left ventricle has a constant short half life of  $4.7 \pm 0.2$  seconds. The half life is not related to either the degree or the duration of increased work.  $\alpha$ ,  $\beta$  and adenosine inhibitors do not alter this half life. Coronary  $\alpha$ -adrenergic vasoconstriction is dominant in the neurogenic control of coronary circulation in response to the increase of afterload of left ventricle.

## **CHAPTER 6**

### **RESULT 2 – RELATIONSHIPS BETWEEN CORONARY RESISTANCE AND METABOLIC PARAMETERS**

## 6.1 RESULTS

During the analysis of the experimental results, it was found that many parameters, such as pH, pCO<sub>2</sub>, pO<sub>2</sub> and SO<sub>2</sub>, had changed following the recovery in coronary blood flow and coronary resistance after the withdrawal of systemic hypoxia. Therefore, the meaningful study of decay of the response was totally prohibited. Thus, I have directed the investigation to analysis of the correlation between coronary resistance and other parameters.

### 6.1.1 Hemodynamic Variables and Assessment of Systemic Hypoxia

There was no detectable deterioration of myocardium during either the normoxic oxygen supply or the low oxygen gas supply. In the uncontrolled experimental model, systemic hypoxia caused by different types of mixed gases was studied. Two typical phenomena were observed and are shown in Figure 6.1 and Figure 6.2. The following abbreviations are used:

<b>cqm</b>	mean coronary blood flow (mls/min)
<b>cr</b>	mean calculated diastolic coronary resistance (mmHg min/ml)
<b>pqm</b>	mean pulmonary blood flow (litres/min)
<b>HR</b>	heart rates (beats/min)
<b>s.aop</b>	systolic proximal aortic pressure (mmHg)
<b>d.aop</b>	diastolic proximal aortic pressure (mmHg)
<b>s.LVP</b>	systolic left ventricular pressure (mmHg)
<b>LVDP</b>	diastolic left ventricular pressure (mmHg)
<b>pH(A)</b>	pH – arterial blood from proximal aorta
<b>pCO<sub>2</sub>(A)</b>	pCO <sub>2</sub> – arterial blood from proximal aorta (mmHg)
<b>pO<sub>2</sub>(A)</b>	pO <sub>2</sub> – arterial blood from proximal aorta (mmHg)
<b>SO<sub>2</sub>(A)</b>	% oxygen saturation of arterial blood from proximal aorta
<b>pH(V)</b>	pH – venous blood from coronary sinus
<b>pCO<sub>2</sub>(V)</b>	pCO <sub>2</sub> – venous blood from coronary sinus (mmHg)
<b>pO<sub>2</sub>(V)</b>	pO <sub>2</sub> – venous blood from coronary sinus (mmHg)
<b>SO<sub>2</sub>(V)</b>	% oxygen saturation of venous blood from coronary sinus

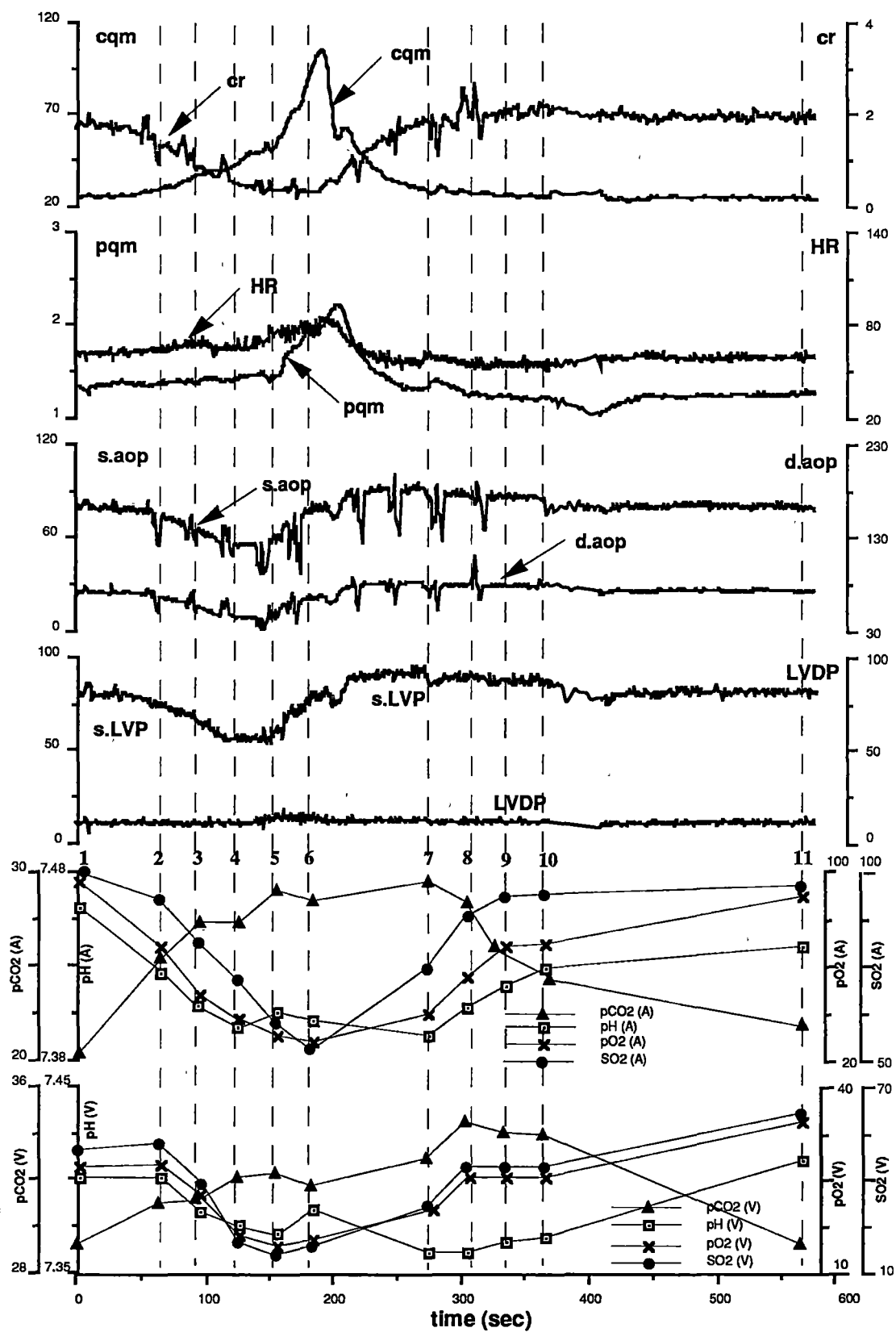


Fig. 6.1: One type of change in hemodynamic variables and assessments due to hypoxia. (Data from experiment 90.10.16, type III intervention for 150 seconds, see text for details). The broken lines in aop and cr were caused by sampling.

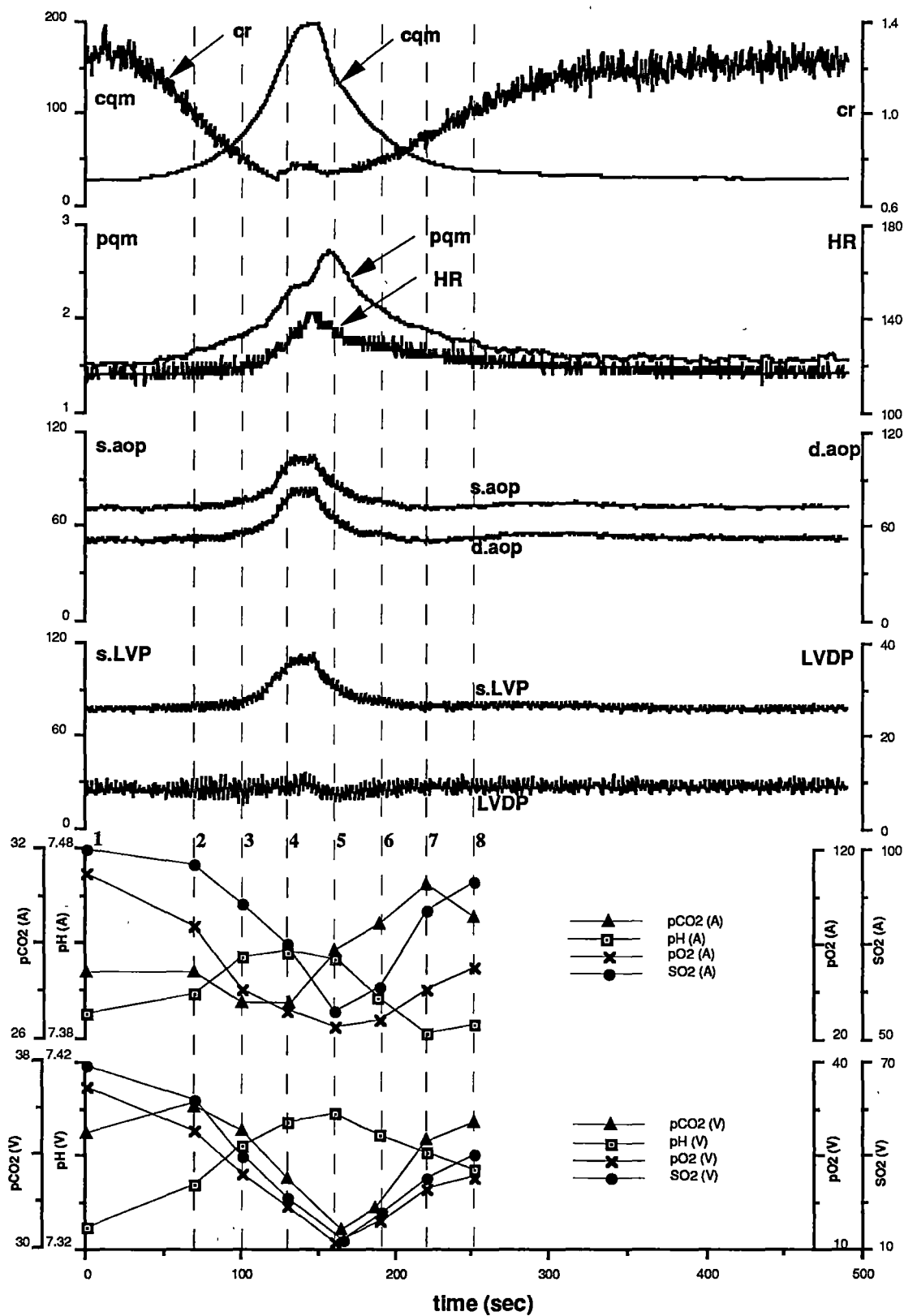


Fig 6.2: One type of change in hemodynamic variables and assessments due to hypoxia (Data from experiment 90.9.25, type I intervention for 120 seconds, see text for detail).

In Figure 6.1, 11 blood samples were taken: sample **1** - baseline before intervention; **2** - 60secs after low oxygen supply; **3** - 90secs after intervention; **4** - 120secs after intervention; **5** - 150secs after intervention (at this point intervention was withdrawn); **6** - 30secs after withdrawal; **7** - 90secs after withdrawal; **8** - 120secs afterwards; **9** - 150secs afterwards; **10** - 180 secs afterwards; **11** - after all the hemodynamic variables recovered to the stable state.

The response to the applied intervention (systemic hypoxia) included:

- **cqm** increased and **cr** decreased. This response lasted more than 30secs after the withdrawal of intervention before returning toward the control values.
- cardiac output, represented by **pqm**, increased and **HR** also increased.
- both aortic pressure and left ventricular pressure continuously decreased initially, then increased before the intervention was withdrawn and stayed nearly stable while **cqm** and **cr** recovered toward the baseline.
- **LVDP** increased while **s.LVP** and **aop** decreased.
- **pH** in both artery and vein decreased with hypoxia and then gradually recovered toward the baseline. There is some delay of response in **pH** after withdrawal of intervention.
- **pCO<sub>2</sub>** in both artery and vein continuously increased, until 120secs after the withdrawal of the intervention before recovering toward the baseline.
- **pO<sub>2</sub>** and **SO<sub>2</sub>** in both arterial and venous blood were shifted consistently, especially in venous. They decreased initially and remained low for at least another 30 secs after the withdrawal of intervention and then gradually recovered to the control values.

In Figure 6.2, 8 blood samples were taken: **1** - control values; **2** - 60secs after intervention; **3** - 90secs after stimulus; **4** - 120secs after stimulus (at this point, the intervention was withdrawn); **5** - 30secs after withdrawal; **6** - 60secs after

withdrawal; 7 - 90secs afterwards; 8 - 120secs afterwards. The differences of reaction from Figure 6.1 to 6.2 are:

- The pressures in **s.aop**, **d.aop**, **s.LVP** and **L VDP** continuously increased even after the withdrawal of stimulus, then decreased toward the baseline before the measured lowest **pO<sub>2</sub>** was reached.
- **pH** increased first, then decreased; **pCO<sub>2</sub>** decreased, then increased.

There were 7 sheep using mixed gases (type III<sup>1</sup>) as stimulus, 3 used type I and II as stimulus. Those using type III all showed the first type of response to hypoxia as Figure 6.1. However, other sheep using type I and II all demonstrated the reaction as shown in Figure 6.2.

#### 6.1.2 The Correlation between Coronary Resistance and Parameters before and after Adenosine Inhibition

Following the results shown in Figure 6.1 and 6.2, the correlations between the coronary resistance (**cr**) and various properties (**pH**, **pCO<sub>2</sub>**, **pO<sub>2</sub>**, **SO<sub>2</sub>**) of both proximal aortic blood and coronary sinus and myocardial oxygen consumption (**MVO<sub>2</sub>**) in 8 sheep before and after adenosine inhibition<sup>2</sup> were determined by fitting the equation:

$$cr = a + bx$$

(where **x** is the other parameter). The complete data sets are set out in appendix D. Table 6.1 summarizes the values, **n** (sample size), **a** (intercept), **b** (gradient) and **r** (correlation coefficient), amongst all the variables in 8 sheep. Sheep 3 and 7 used type I and II as intervention, others used type III. The statistical significance was also

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<sup>1</sup>Gas mixtures are listed in section 4.4.2.

<sup>2</sup>Adenosine blocking effect by 8-phenyltheophylline will be shown in chapter 8.

determined from the value of **r** and **n** [Hoel, 1976]. The results in Table 6.1 may be summarized as follows:

- There was a poor relationship between **cr** and **pH(A)**, except in sheep 4 and 6 in which there was a positive slope. There were three negative and five positive slope in all.
- There was a poor relationship between **cr** and **pCO<sub>2</sub>(A)**.
- Good correlations (statistical significance:  $p \leq 0.05$ ) were found between **cr** and oxygen (**pO<sub>2</sub>**; **SO<sub>2</sub>**) from both **artery** and **vein** in all sheep.
- The relationship between **cr** and venous **pH** and **pCO<sub>2</sub>** varied from sheep to sheep with no consistent response.
- Statistically meaningful correlation was found between **cr** and **pCO<sub>2</sub>(V)** in 2 sheep in which one showed a negative slope and the other one a positive slope.

**After adenosine blockade:**

- There was a poor correlation between **cr** and **pH(A)**.
- Poor correlation was obtained between **cr** and **pCO<sub>2</sub>(A)**.
- There was a good correlation between **cr** and **pO<sub>2</sub>(A)**. 2 out of 7 were statistically significant.
- Very good correlations (statistically significant) were found between **cr** and **SO<sub>2</sub>(A)** in all sheep. The relationship was improved from control.



**Table 6.1: Linear regression analysis between cr and other parameter amongst 8 sheep**

Condition	Parameter	Results	Sheep No.								
			1	2	3	4	5	6	7	8	
Control		n	11	8	7	10	7	8	12	7	
Artery	pH (A)	a	-148.9	-53.4	9.26	-86.8	-73.1	-67.6	63.5	11.8	
		b	20.27	7.3	-1.12	11.6	9.61	9.07	-8.19	-1.40	
		r	0.31	0.35	0.01	0.68*	0.73	0.94†	0.22	0.001	
	pCO2 (A)	a	5.25	3.9	1.56	2.79	3.92	3.42	-2.50	9.74	
		b	-0.15	-0.02	-0.02	-0.10	-0.14	-0.11	0.14	-0.26	
		r	0.32	0.51	0.10	0.54	0.64	0.88†	0.18	0.88†	
	pO2 (A)	a	-0.08	0.39	0.54	0.46	0.39	0.32	0.29	-0.01	
		b	0.02	0.02	0.09	0.09	0.01	0.09	0.01	0.05	
		r	0.65*	0.73*	0.74	0.57	0.64	0.73*	0.71†	0.95†	
	SO2 (A)	a	-2.1	0.22	0.46	-0.19	-1.48	-1.65	-0.62	0.14	
		b	0.04	0.01	0.06	0.01	0.03	0.03	0.02	0.02	
		r	0.77†	0.81*	0.60*	0.59	0.81*	0.60	0.65*	0.98†	
	Venous	pH (V)	a	-53.3	-24.7	52.33	-149.7	-135.4	-100.7	91.6	-386.3
			b	7.42	3.47	-6.94	20.0	18.0	13.5	-11.9	52.2
			r	0.03	0.03	0.72	0.71*	0.98†	0.72*	0.63*	0.23
pCO2 (V)		a	-0.21	3.12	-0.77	4.41	4.27	4.62	-3.19	4.86	
		b	0.05	-0.04	0.04	-0.15	-0.13	-0.14	0.14	-9.22	
		r	0.02	0.07	0.70	0.34	0.88†	0.66	0.59*	0.01	
pO2 (V)		a	-0.99	-0.02	0.46	0.39	-1.03	0.11	-0.06	0.09	
		b	0.1	0.07	0.02	0.02	0.11	0.03	0.05	0.11	
		r	0.73†	0.84*	0.57	0.34	0.84*	0.27	0.66*	0.84†	
SO2 (V)		a	-0.25	0.41	0.58	0.47	-0.31	0.04	0.17	0.68	
		b	0.04	0.03	0.008	0.01	0.03	0.02	0.02	0.05	
		r	0.72*	0.86*	0.61*	0.45	0.89†	0.41	0.60*	0.85†	
MVO2		a	2.62	1.44	1.41	1.98	1.88	2.22	1.72	-0.86	
		b	-0.05	-0.01	-0.03	-0.02	-0.07	-0.06	-0.03	-0.02	
		r	0.68*	0.19	0.55	0.92†	0.49	0.76*	0.59*	0.71*	
After ADO inhibition											
Artery		n	6	6	10	8	7	7	8		
pH (A)		a	-75.2	-29.07	81.27	-62.8	-140.8	-47.9	14.4		
		b	10.3	3.96	-10.88	8.54	18.9	6.43	-1.79		
		r	0.62	0.65	0.51	0.35	0.85*	0.56	0.06		
pCO2 (A)		a	2.59	1.85	0.03	2.95	4.03	2.77	-0.03		
		b	-0.07	-0.04	0.02	-0.09	-0.10	-0.09	0.03		
		r	0.82*	0.65	0.02	0.38	0.84*	0.61	0.08		
pO2 (A)		a	-0.04	0.81	0.43	0.69	-0.01	0.66	0.32		
		b	0.01	0.02	0.04	0.01	0.03	0.06	0.01		
		r	0.89*	0.57	0.56	0.67	0.91†	0.53	0.66		
SO2 (A)		a	-0.54	-0.08	-0.56	-0.13	-1.44	-2.65	-0.91		
		b	0.01	0.02	0.01	0.02	0.04	0.04	0.02		
		r	0.82*	0.81*	0.79*	0.78*	0.96†	0.96†	0.76*		
MVO2		a	1.16	1.31	1.10	2.4	1.31	2.23	0.79		
		b	-0.02	-0.02	-0.02	-0.003	-0.02	-0.07	0.04		
		r	0.73	0.20	0.83*	0.82*	0.20	0.90†	0.14		

n - number of data points; a - intercept; b - slope; r - correlation coefficient; \* - significantly associated ( $p \leq 0.05$ ); † - significantly associated ( $p \leq 0.01$ ). ADO - adenosine

Figure 6.3 exhibits the correlation between percentage change of coronary resistance and percentage change of proximal aortic oxygen saturation in 8 sheep before and after adenosine inhibition by 8-phenyltheophylline. Other parameters did not show a statistically meaningful correlation.

The slopes from data A and B in Figure 6.3 were compared by calculating the statistic calculated  $T$  which is distributed as a Student's  $t$  with *data A size + data B size - 4* degree of freedom<sup>1</sup>. However, in this case,

**Slope in graph B = + 1.57**

**Slope in graph A = + 0.93**

**$T = 3.107$**

From student's  $t$  table:

**$t = 2.617$ , when  $df = 120$ ,  $p = 99.5\%$**

**so  $T > t$ ,**

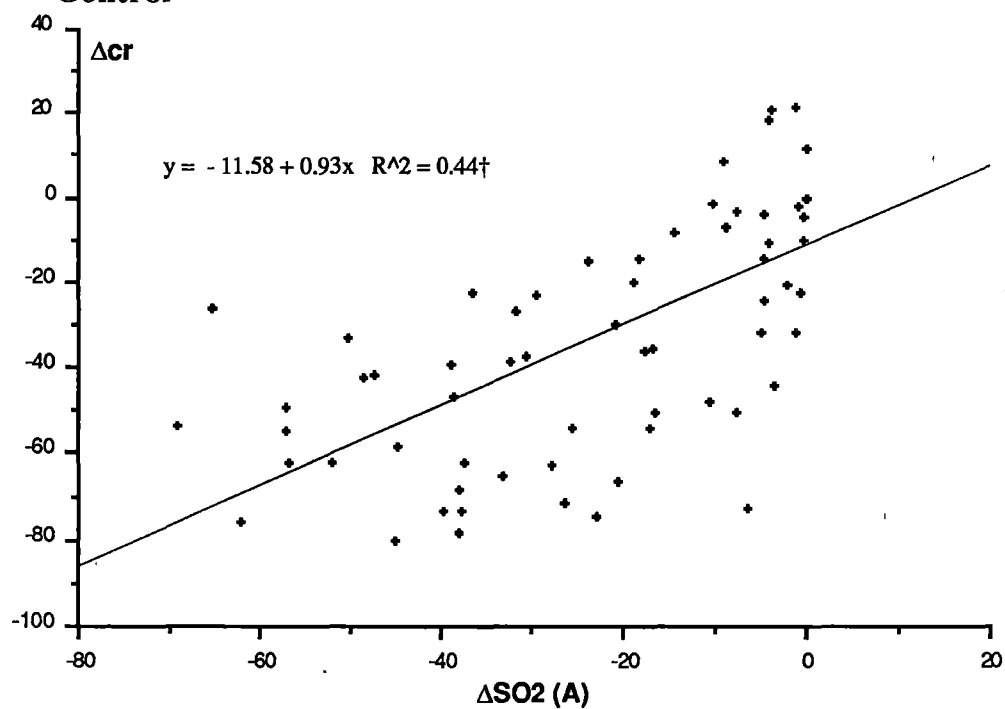
and there is less than 0.5% possibility for slope A and slope B to be the same, *ie* slope B is significantly steeper than slope A.

In summary, there is a linear relationship between coronary resistance and  $pO_2$ ,  $SO_2$  which is better and steeper after adenosine blockade, *ie* after adenosine blockade, a change of oxygen concentration in the artery causes a relatively larger change of coronary resistance than before.

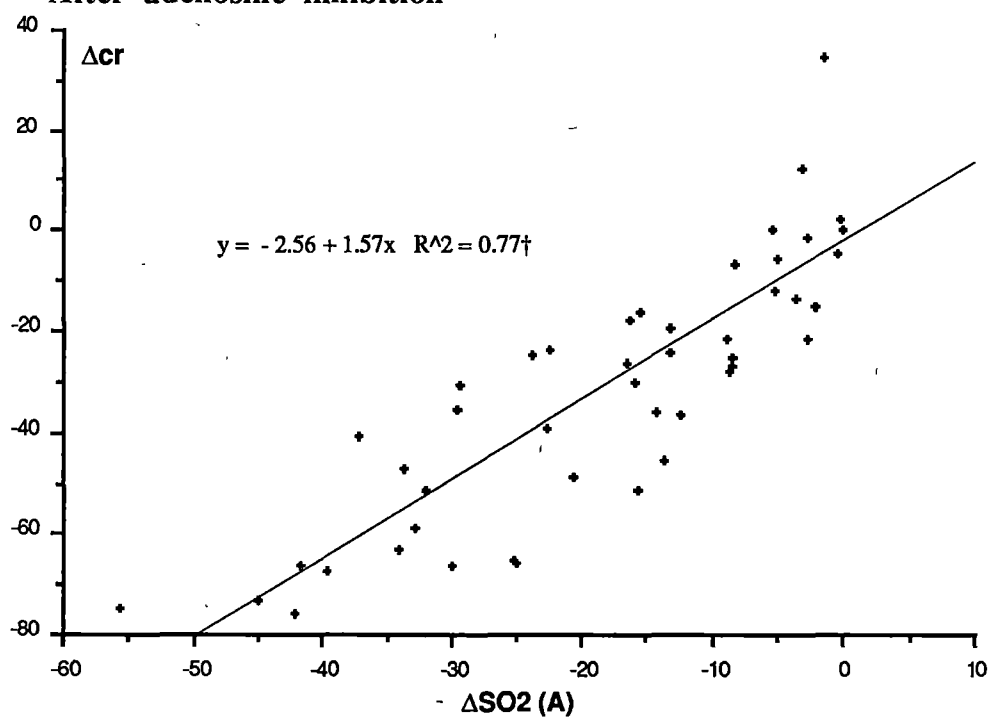
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<sup>1</sup>Refer to section 4.3.4.

**A: Control**



**B: After adenosine inhibition**



**Fig. 6.3:** The correlation between the percentage change of coronary resistance ( $\Delta cr$ ) and the percentage change of arterial oxygen saturation ( $\Delta SO_2(A)$ ) before (A) and after (B) adenosine inhibition. (70 and 52 data points from 8 sheep,  $^\dagger$  - statistically significantly associated,  $p \leq 0.01$ )

## 6.2 DISCUSSION

In the current study, the calculated coronary resistance (**cr**) was employed to investigate the effect of systemic hypoxia on coronary vasodilation before and after 8-phenyltheophylline. Systemic hypoxia has been observed to augment cardiac sympathetic nerve activity in the canine myocardium [Feigl, 1983]. This response was also shown in my preparation (Figure 6.1 and 6.2). The hypoxia causes an increase of the aortic pressure and an increased perfusion pressure for coronary circulation. So, using **cr** rather than coronary blood flow (**CBF**) in the analysis can minimize this effect.

The difference between Figure 6.1 and 6.2 may be caused by the different mixed gases used as intervention because there is an extra 5%  $\text{CO}_2$  in the gases for the Figure 6.1 experiments. This extra  $\text{CO}_2$  has a direct negative inotropic effect on the heart [Williamson, 1975], which causes an initial decrease in proximal aortic pressure and left ventricular pressure until the sympathetic effect overcomes it. Thus, gas type I and II shows a greater sympatho-adrenergic response than type III.

In the study of correlation between **cr** and the other parameters, 2 out of 8 sheep between **cr** and **pH** in arterial blood showed significant association. In both cases,  $\text{pH} > 7.5$ : Thus the systemic hypoxia only decreased the severity of alkalosis which may be caused by over-ventilation (both showed low arterial  $\text{pCO}_2$ ), and did not cause acidosis. However, the rest showed acidosis caused by hypoxia and did not show significant association with **cr**. Thus, the 2 correlations observed should not lead to the conclusion that arterial acidosis plays a role in this hypoxia induced vasodilation.

Scheuer [1968] used hyperventilation and bicarbonate infusion to increase **pH** (7.35  $\rightarrow$  7.87) under nearly constant arterial  $\text{pCO}_2$ : this did not induce significant changes in **CBF** and  $\dot{\text{MVO}}_2$ . However, they did not calculate the **cr**. In my study,

although in 4 sheep, **cr** correlated with **pH** in coronary sinus (CS) blood, two of these sheep were the same sheep which showed the correlation between **cr** and **pH(A)**. The other two showed negative slopes. If the values of acid-base and blood gases in CS indicate the value in myocardium, my results do not support the hypothesis that the severity of acidosis in the myocardium has an effect on the determination of **cr**.

The present results do not completely exclude **pH** as an agent in coronary vasodilation, because the stimuli did not cause **pH** to fall below 7.2. At this **pH** value, other workers discovered that has an independent effect on coronary vasculature resistance in isolated rabbit hearts [Clancy, 1975] and in closed-chest anaesthetized dogs [Tarnow, 1975]. However, this does not support **pH** as playing a role in hypoxia induced physiological vasoregulation of CBF.

**pCO<sub>2</sub>(A)** showed correlation with **cr** in 2 sheep – neither of which had a correlation with **pH(A)**. Neither case used type I or II gases as the intervention which elicits a greater sympathetic response. This result is consistent with previous findings – increased **pCO<sub>2</sub>** is associated with CBF in some experiments but not in others. Van den Bos and co-workers [1979] studied the effect of high **pCO<sub>2</sub>** on CBF by using adrenergic  $\beta$ -receptor blockade to stabilize **MVO<sub>2</sub>** and found no change in CBF when **pCO<sub>2</sub>(A)** was doubled. They concluded that the coronary vasodilation observed during hypercapnia was due to an adrenergic effect and not to a direct effect of CO<sub>2</sub>. If their conclusion is true, those sheep (sheep 3 and 7) showing a greater sympatho-adrenergic response to extra 5% CO<sub>2</sub> in mixed gases should exhibit the better correlation with **cr** than others. However this was not the case. The current approach could not determine the role of arterial **pCO<sub>2</sub>** in coronary regulation. The CO<sub>2</sub> production in myocardium, represented by **pCO<sub>2</sub>(CS)**, was independent of **cr** in most sheep. However, the effect of myocardial CO<sub>2</sub> on vascular smooth muscle could

not be excluded because the effect may be masked by other concomitant changes which cause more dominant changes in **cr**.

Both **pO<sub>2</sub>(A)** and **SO<sub>2</sub>(A)** showed a good correlation with **cr** in all the sheep. This makes it impossible to reject the association between arterial oxygen and **cr** in response to systemic hypoxia although other factors also varied under this condition. By what mechanism is arterial oxygen related to **cr**? In section 3.2, it was noted that systemic hypoxia undoubtedly caused the increase in cardiac sympathetic nerve activity, in the current preparation shown by the increase of heart rate, cardiac output and aortic pressure.  $\alpha_1$ -adrenergic coronary vasoconstriction appeared to be dominant in the neural control of **CBF** during hypoxia [Grice, 1987]. This would lead us to suppose the vasodilation observed in this study was purely due to local regulation. Since **pO<sub>2</sub>** in **CS** did not fall below 8mmHg, which is the critical value for mitochondria [Berne, 1980], it is unlikely this vasodilation was caused by direct effect of oxygen on resistance vessel tone<sup>1</sup>. Amongst all the putative candidates found to be responsible for hypoxia induced coronary vasoregulation at one time or another, adenosine appears to have most support although there is no direct evidence for this. If adenosine plays an important role in hypoxic vasodilation, it is logical to expect that after using a potent adenosine blocker (8-phenyltheophylline) [Smellie, 1979; Griffith, 1981] there would be a poorer correlation between arterial oxygen and **cr** and the change of arterial oxygen should elicit less change in **cr**. However, my data show the opposite result. It suggests that adenosine does not play an important role in hypoxia induced-vasoregulation. This conclusion is not consistent with recent work done in Merrill's laboratory, where they show both adenosine deaminase and 8-Phenyltheophylline attenuated canine coronary vasodilation during systemic hypoxia

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<sup>1</sup>Refer to section 3.2.3.

[Wei, 1988; 1989]. Their results may be influenced by their non-physiological approach in which they used a controlled-flow system for investigation.

Because the open-chest hemodynamic uncontrolled sheep was used as an experimental model, baseline hemodynamic variables would be different from sheep to sheep, and systemic hypoxia undoubtedly would influence neural control, which would cause changes in heart rate, cardiac output and aortic pressure. All the parameters including pH, pCO<sub>2</sub>, pO<sub>2</sub>, SO<sub>2</sub> and MVO<sub>2</sub> are varied together during systemic hypoxia. All this complicated the interpretation of the response of coronary blood flow and coronary resistance to systemic hypoxia.

### **6.3 CONCLUSION**

The current study is not conclusive in determining which of the putative vasodilators have an effect on the coronary vasodilation due to systemic hypoxia because of the concomitant changes in other variables during intervention. However, it shows that arterial oxygen is strongly correlated with coronary vascular resistance. It also suggests that adenosine is unlikely to act as the transmitter between myocardial hypoxia and coronary vascular smooth muscle relaxation.

## **CHAPTER 7**

### **RESULT 3 – CONTINUOUS MEASUREMENTS OF CORONARY SINUS OXYGEN SATURATION DURING INCREASED CARDIAC WORK AND SYSTEMIC HYPOXIA**



## 7.1 RESULTS

### 7.1.1 Change in $\text{SO}_2$ from Coronary Sinus due to Increased Cardiac Work

Figure 7.1 shows a typical set of traces from the polygraph including  $\text{SO}_2$  from OXIMETER.

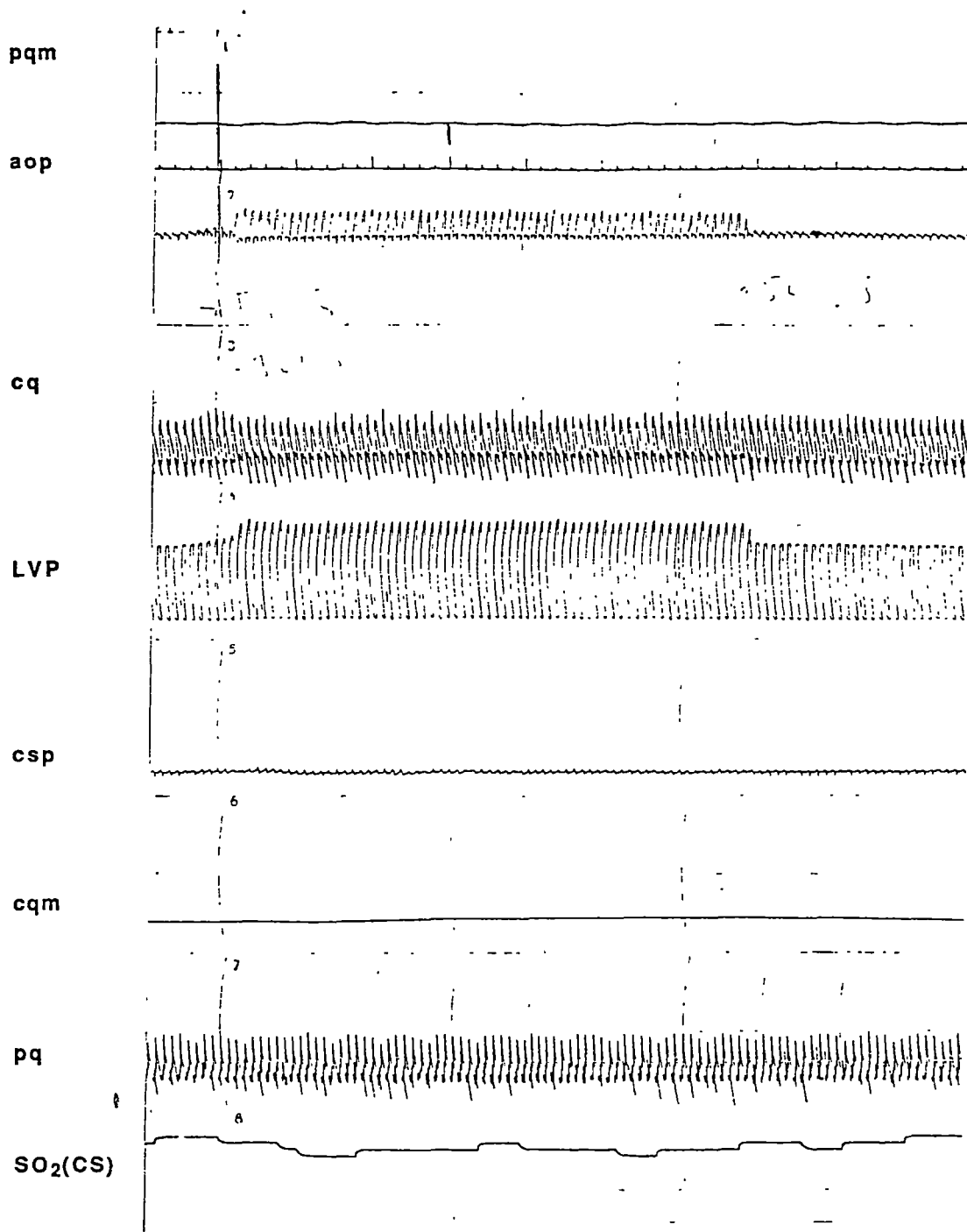
There was no consistency in the response of  $\text{SO}_2$  during increased cardiac work. This result was surprising in view of our anticipation of increased work causing an increase of oxygen uptake. There were three types of change in coronary sinus  $\text{SO}_2$  in response to increased cardiac work caused by increased proximal aortic pressure in this study. They were:

- a) Increase in  $\text{SO}_2$  (Figure 7.2)
- b) Decrease in  $\text{SO}_2$  (Figure 7.3)
- c) Nearly stable  $\text{SO}_2$  (Figure 7.4)

Figure 7.2 shows that  $\text{SO}_2(\text{CS})^1$  increased from 68% to 73% as the aortic constriction was applied, fluctuated above the control value during intervention and returned to the baseline after the stimulus was withdrawn. In another sheep (Figure 7.3), a decrease of  $\text{SO}_2(\text{CS})$ , from 65% to 60%, was observed in response to aortic constriction. However, in another trial (Figure 7.4) a nearly stable  $\text{SO}_2(\text{CS})$  ( $\pm 1\%$ ) was demonstrated. In these three figures, other hemodynamic variables showed similar responses to those I discussed in chapter 5.

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<sup>1</sup>Abbreviations are listed in glossary.



**Fig. 7.1:** The changes of hemodynamic variables and  $SO_2$  (CS) due to aortic constriction

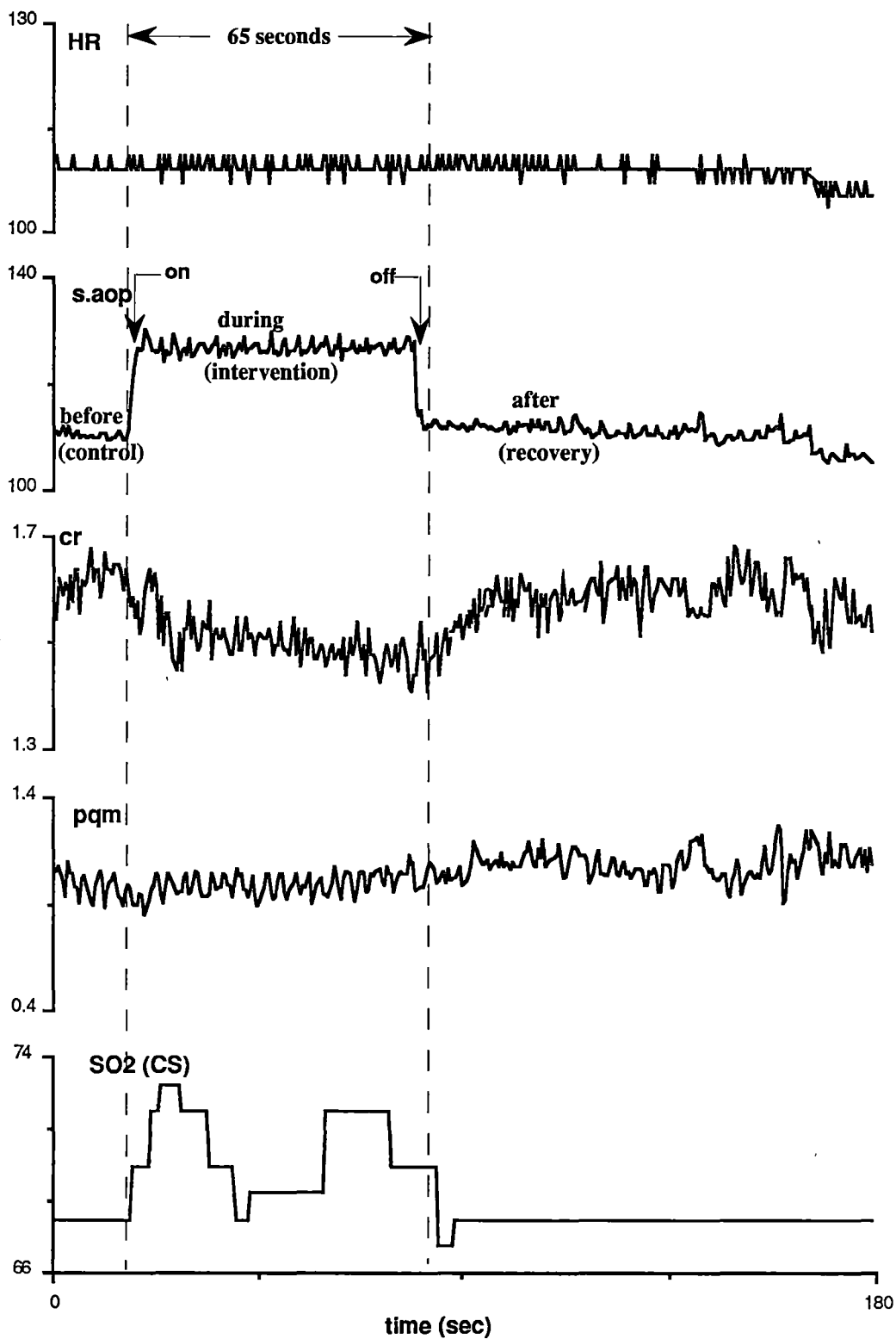


Fig. 7.2: Increase of  $SO_2(CS, \%)$  in response to increased cardiac work. ( $SO_2$  data from polygraph, others from digitized data)

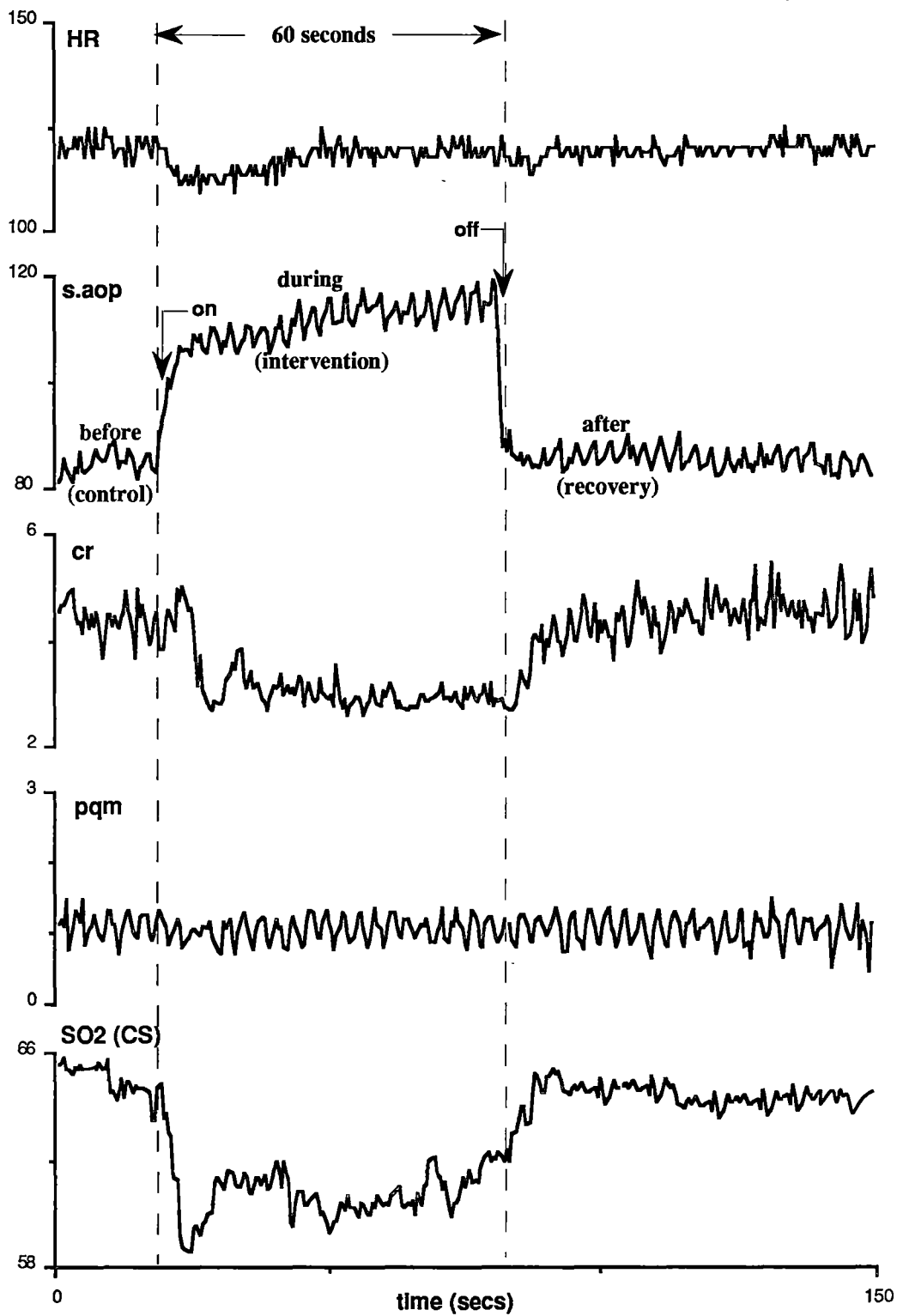


Fig. 7.3: Decrease of  $SO_2(CS, \%)$  in response to increased cardiac work.

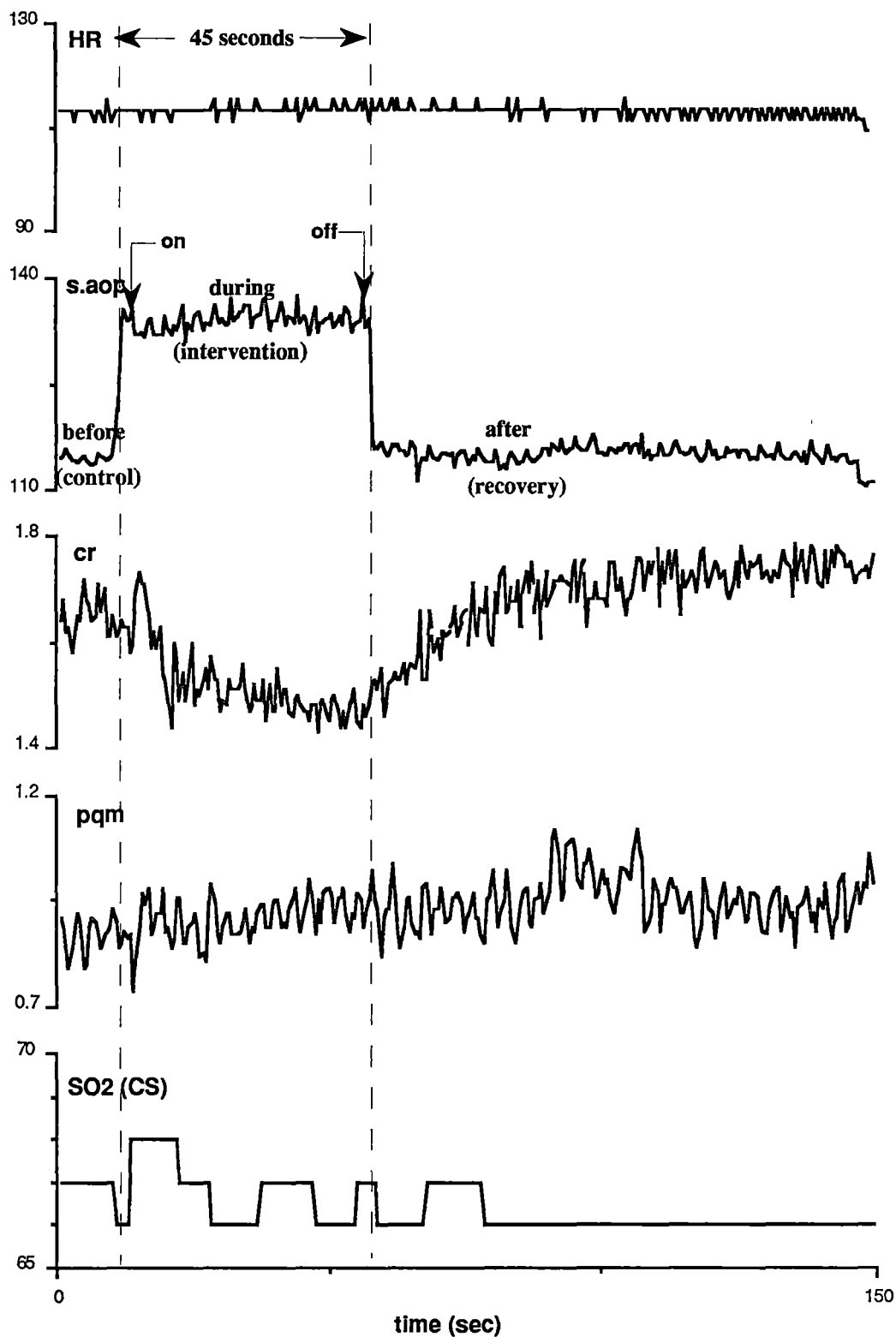


Fig. 7.4: No change in  $SO_2(CS, \%)$  in response to increased cardiac work. ( $SO_2$  data from polygraph, others from digitized data)

Table 7.1 summarized the change of  $SO_2$  (CS) in response to increased cardiac work by employing proximal aortic constriction from seven sheep. Coronary sinus oxygen saturation varied significantly both above and below resting states during intervention.

**Table 7.1:** Summary of changes of  $SO_2$ (CS) in response to increased cardiac work

Sheep	Total Experimental Runs	$SO_2$ (CS) in Response to Aortic Constriction		
		No. of Increase	No. of Decrease	No. of no Change ( $\leq 1\%$ )
1 (20.3.90)	5	2	2	1
2 (27.3.90)	7	4	1	2
3 (7.3.90)	8	4	2	2
4 (28.2.90)	6	1	4	1
5 (salmon)	21	4	14	3
6 (3.4.90)	7	5	0	2
7 (29.6.90)	15	7	6	2
SUM	69	27	29	13

Increase - number of runs in which  $SO_2$  increased during aortic constriction;

Decrease - number of runs in which  $SO_2$  decreased during aortic constriction;

no change - number of runs in which the change of  $SO_2$  was less than 1% during aortic constriction.

Because the Oximeter machine, used for continuously measuring  $SO_2$ , became unservicable, I could not investigate this further *eg* to study the change under  $\alpha$ -adrenergic blockade.

### 7.1.2 Change in $\text{SO}_2$ from Coronary Sinus due to Systemic Hypoxia

Continuous measurement of  $\text{SO}_2$  (CS) was also compared with the changes of hemodynamic variables before, during and after systemic hypoxia shown in Figure 7.5.  $\text{SO}_2$  (CS) declined immediately after mixed gases (Type III) were applied,  $\text{cr}$  also started to decrease, but  $\text{cqm}$  did not increase until  $\text{SO}_2$ (CS) fell below 68%. The most striking results were:

- $\text{cqm}$  returned to the control value much earlier than both  $\text{SO}_2$ (CS) (when  $\text{SO}_2$  was only about 54%) and  $\text{cr}$ .
- When  $\text{SO}_2$  had changed direction toward the baseline (point L),  $\text{cr}$  continue to decrease and  $\text{cqm}$  continue to increase for about 10 seconds, then returned toward the baseline. There was a lag (about 10 seconds) in the response of  $\text{cr}$  and  $\text{cqm}$  to the change of  $\text{SO}_2$ .

Data in Figure 7.5 (1000 heart beats) were used for linear regression analysis between  $\text{cqm}/\text{cr}$  and  $\text{SO}_2$ (CS). Excellent correlation ( $r = 0.91$ ) was found between  $\text{SO}_2$ (CS) and  $\text{cr}$ , which was much more closely associated than  $\text{SO}_2$  (CS) and  $\text{cqm}$  (Figure 7.6). In Figure 7.7, the data from Figure 7.6 has been re-plotted in order to distinguish the two stages – oxygen decreasing and oxygen recovery. A "hysteresis" or a "lag" reaction of  $\text{cqm}$  and  $\text{cr}$  can be seen.

However, the data in Figure 7.8 from the same animal did not show the same lag response of  $\text{cr}$  following change of  $\text{SO}_2$ , although good correlations remained between  $\text{cqm}$ ,  $\text{cr}$  and  $\text{SO}_2$  (Figure 7.9). Figure 7.10 shows two stages – oxygen decreasing and recovery – of response shown in figure 7.9. In another sheep (hypoxia experiment), 4 out of 7 (associated with good condition of sheep) hypoxic runs showed the lag reaction in response to the change of  $\text{SO}_2$  similar to that in Figures 7.5 and 7.6. The ratio of coronary resistance to oxygen saturation in coronary sinus was plotted against time through the whole run before, during and after systemic hypoxia continuously on beat by beat basis in Figure 4.11. This shows the constant

ratio during the period of decreasing oxygen supply, a lag response in  $\text{cr}$  when oxygen level had started to recover toward baseline and finally the ratio falling toward a new stable value.

For the same reason, I was only able to use 3 sheep, of which 2 sheep have 2 runs and 1 sheep has 7 runs, for systemic hypoxia experiments with continuous  $\text{SO}_2$  (CS) measurement.



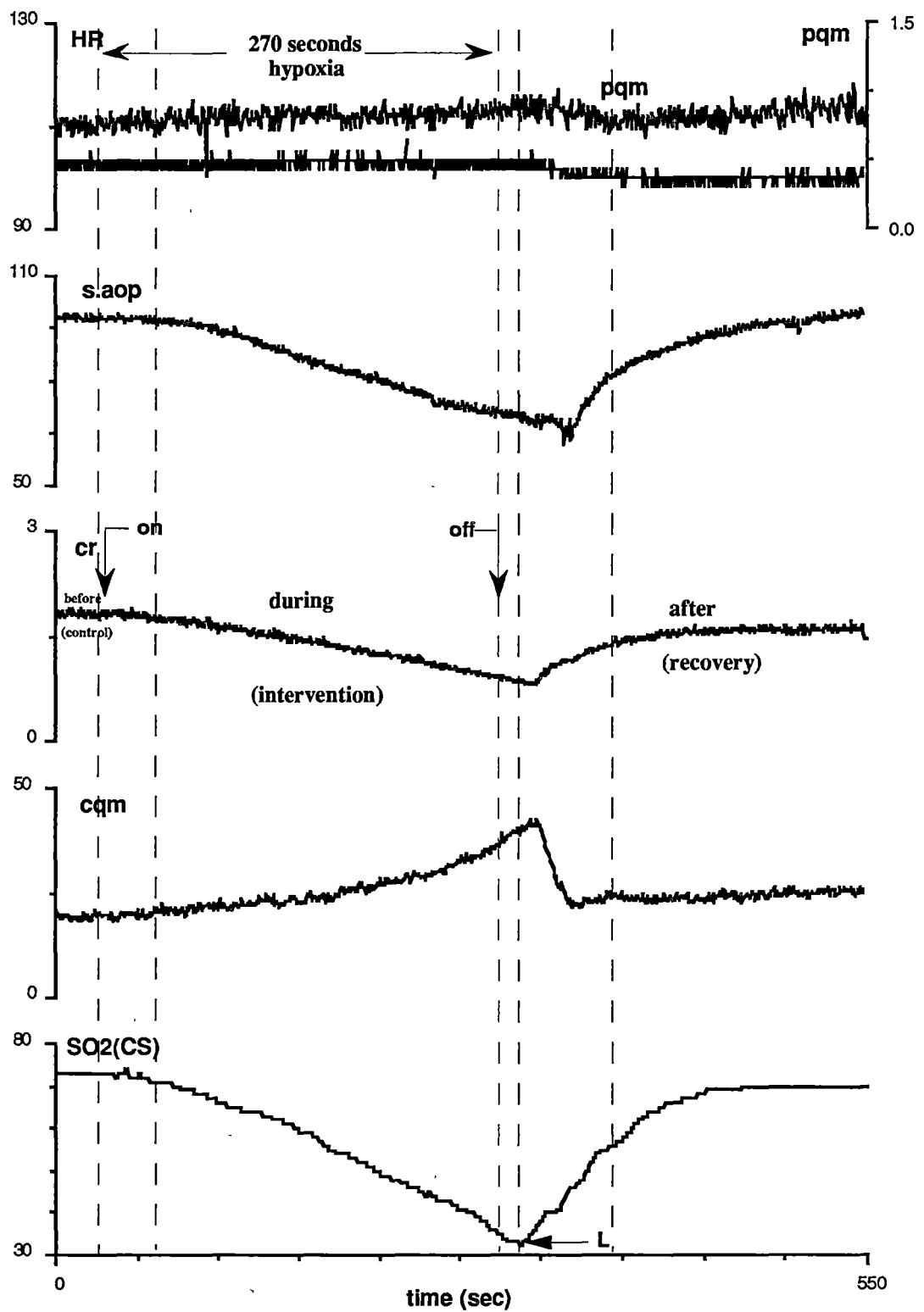
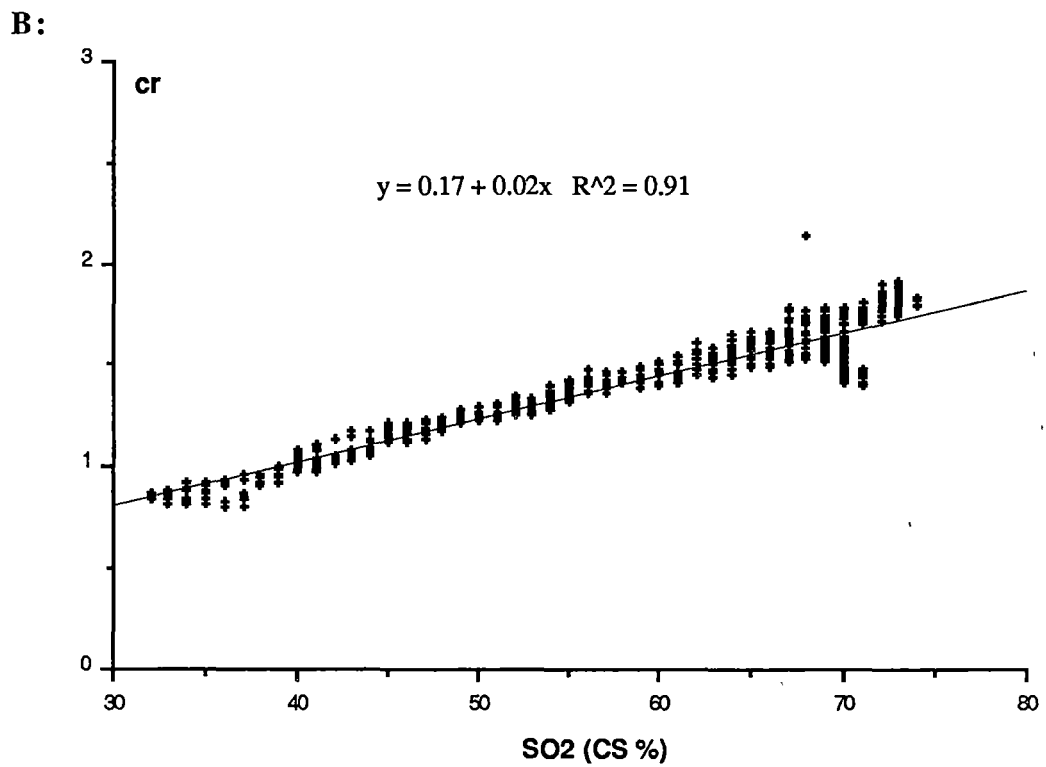
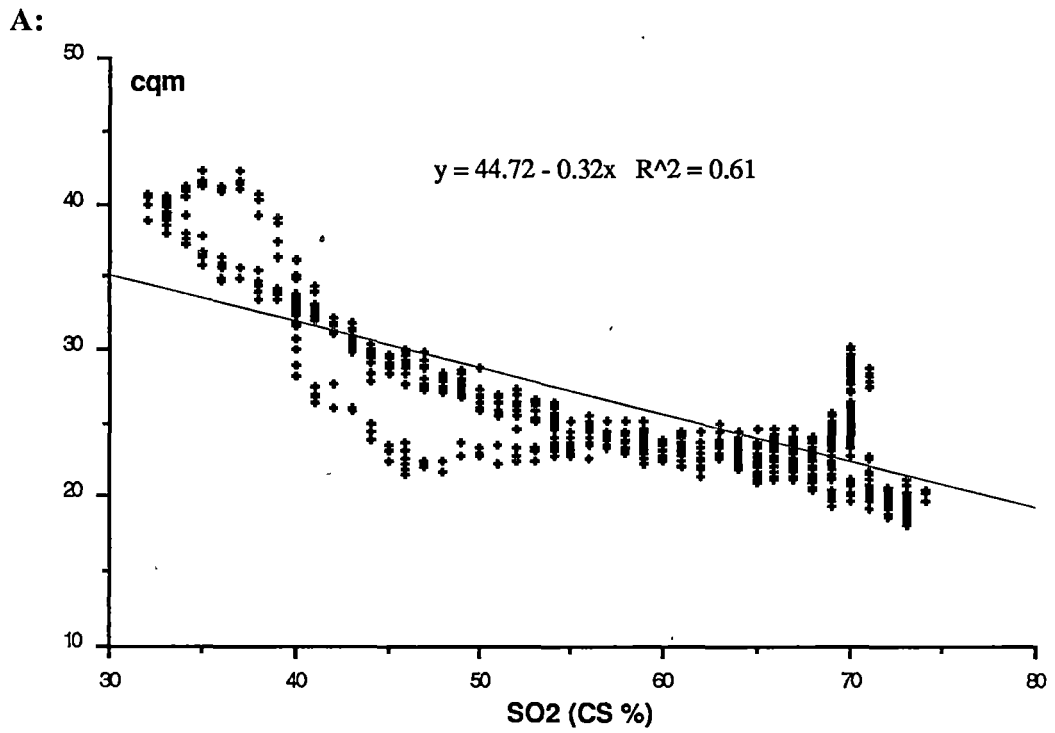
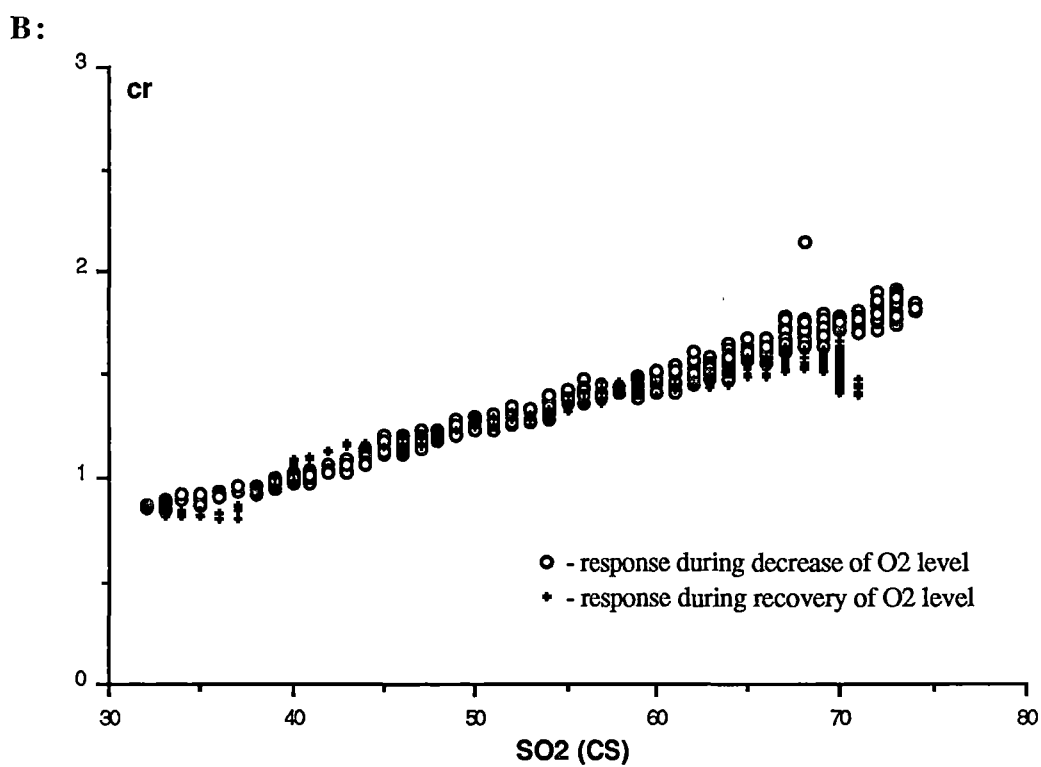
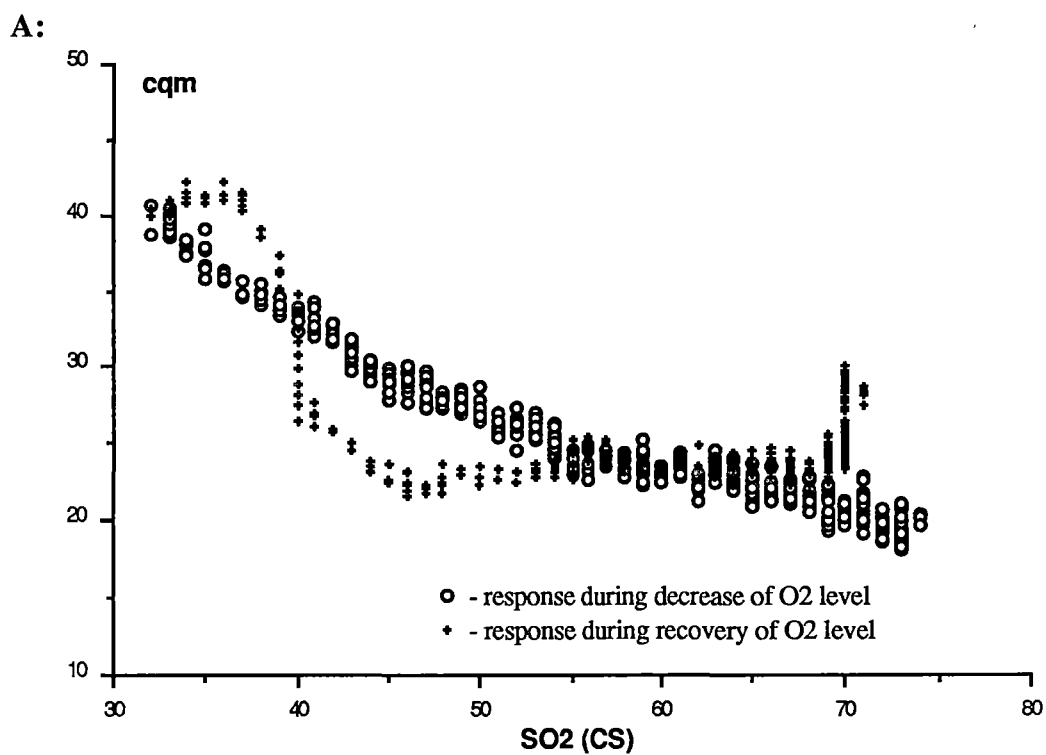


Fig. 7.5: Continuous change in SO<sub>2</sub>(CS, %) and hemodynamic variables in response to systemic hypoxia. (SO<sub>2</sub> data from polygraph, others from digitized data)



**Fig. 7.6:** Correlation between **cqm** (A: mean coronary blood flow), **cr** (B: calculated diastolic coronary resistance) and coronary sinus oxygen saturation (**SO<sub>2</sub>**) continuously on beat by beat basis before, during and after systemic hypoxia (1000 data points - heart beats).



**Fig. 7.7:** Hysteresis or a lag response between **cqm** (A: mean coronary blood flow), **cr** (B: calculated diastolic coronary resistance) and coronary sinus oxygen saturation (SO<sub>2</sub>) continuously on beat by beat basis. Two stages were showed: decrease O<sub>2</sub> supply and recovery in oxygen supply. (same data as Figures 7.5 and 7.6)

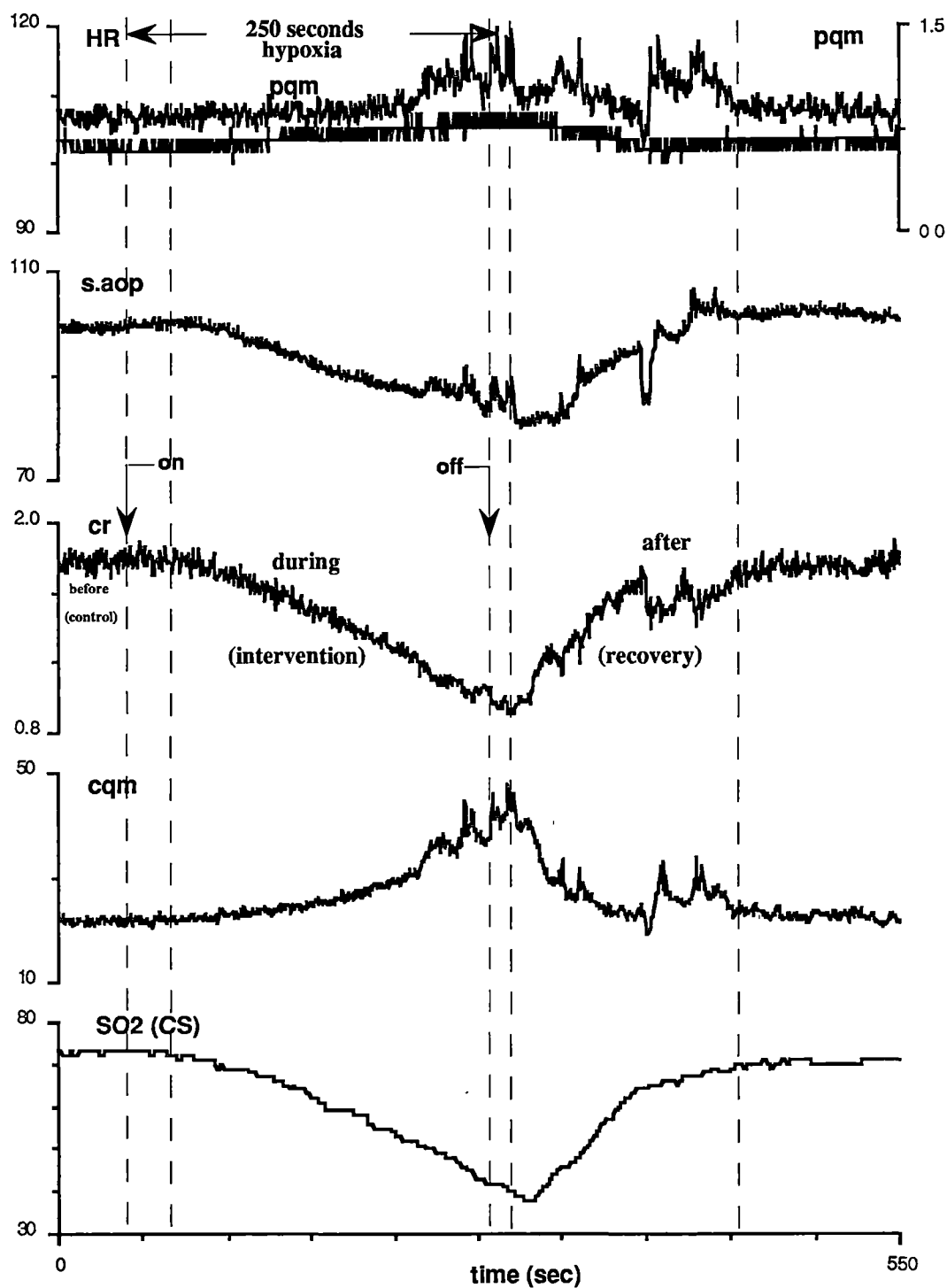
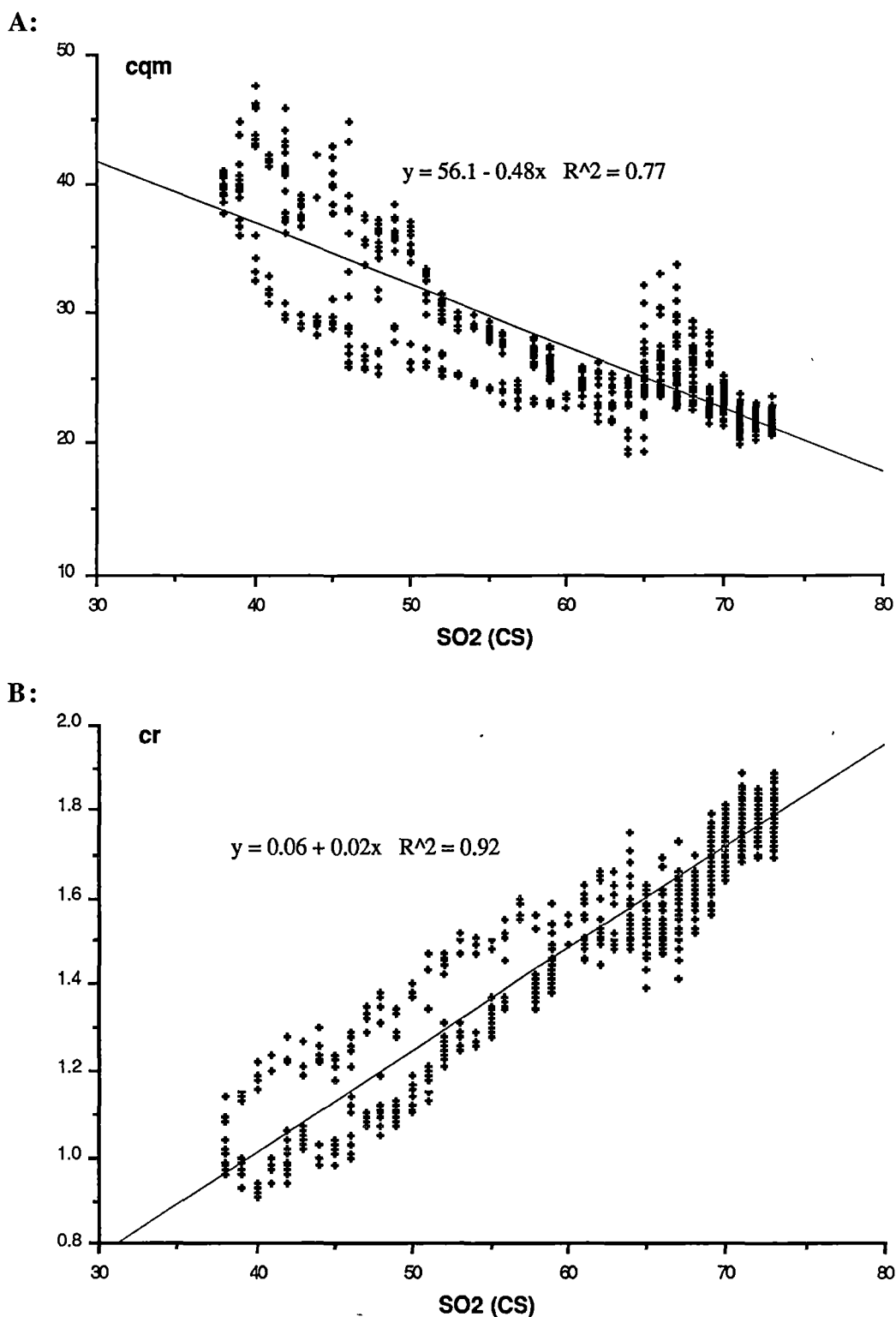
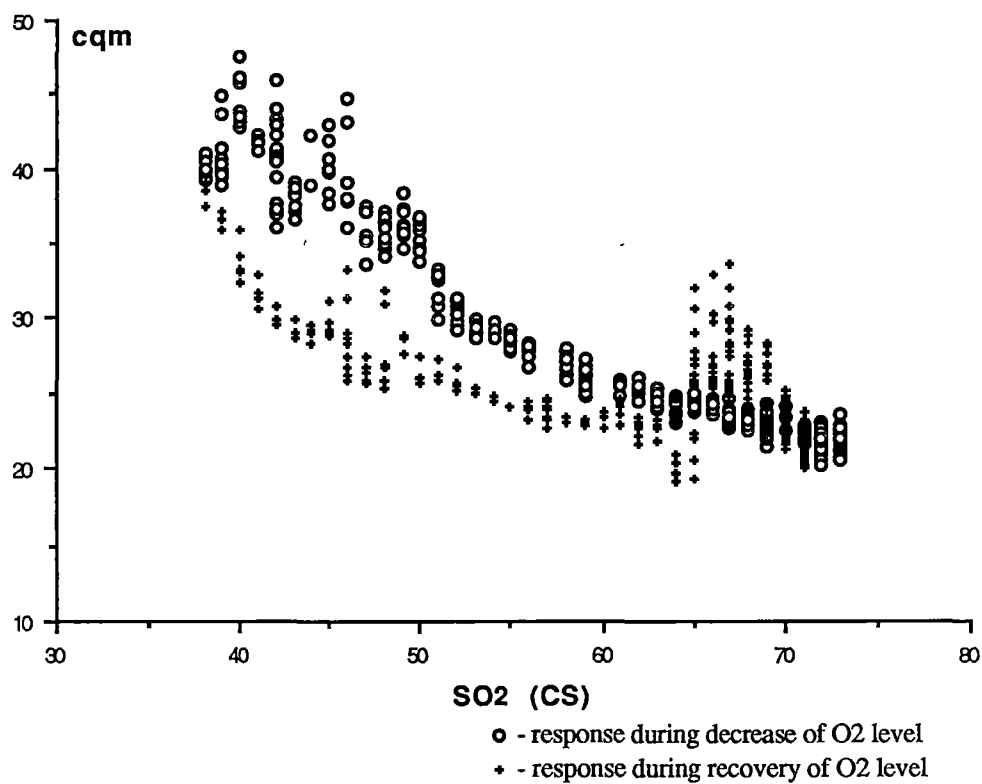


Fig. 7.8: Continuous change in SO<sub>2</sub>(CS, %) and hemodynamic variables in response to systemic hypoxia. (SO<sub>2</sub> data from polygraph, others from digitized data)



**Fig. 7.9:** The correlation between **cqm** (A: mean coronary blood flow), **cr** (B: calculated diastolic coronary resistance) and coronary sinus oxygen saturation (SO2) continuously on beat by beat basis before, during and after systemic hypoxia. (1008 data points - heart beats)

A:



B:

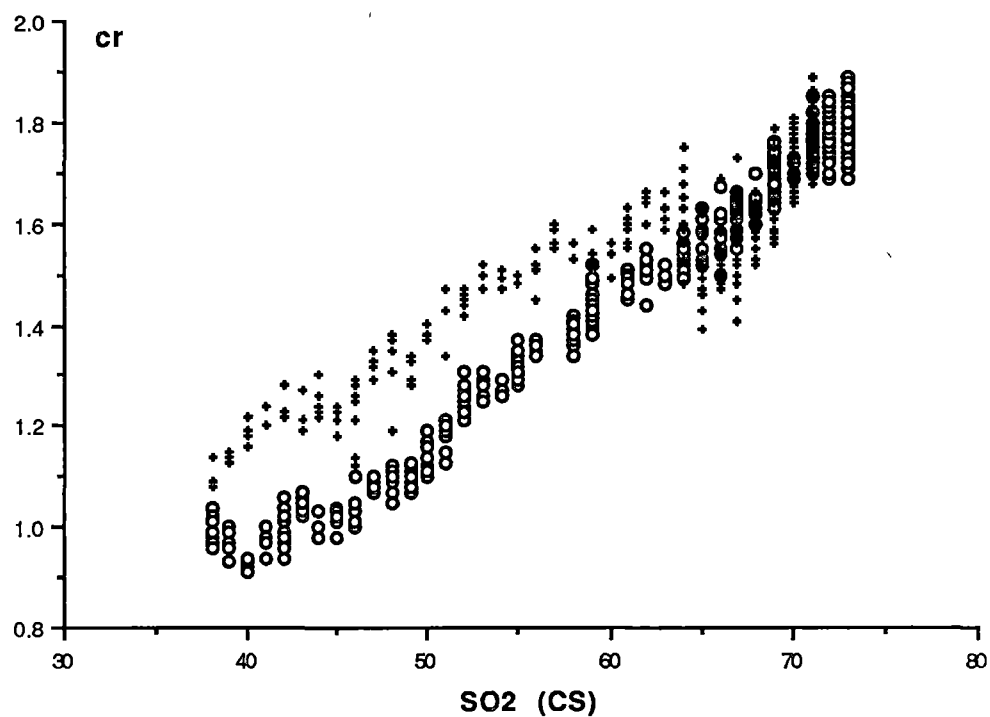


Fig. 7.10: No hysteresis or a lag response was showed between  $cqm$ ,  $cr$  and coronary sinus oxygen saturation ( $SO_2$ ) continuously on beat by beat basis. Two stages were showed: decrease  $O_2$  supply and recovery in oxygen supply. (same data as Figures 7.9)

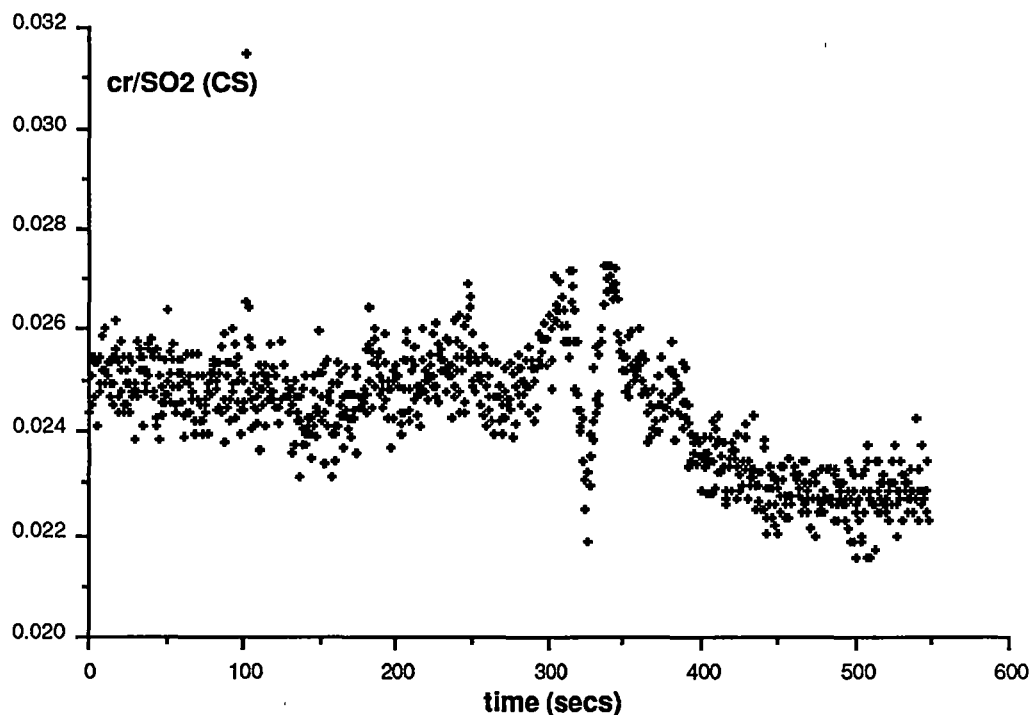


Fig. 7.11: The ratio of coronary resistance and coronary sinus oxygen saturation continuously on beat by beat basis before, during and after systemic hypoxia. (same data as Figure 7.5)

## 7.2 DISCUSSION

Aortic constriction causes the myocyte to do more work in order to overcome the extra resistance due to the augmentation of proximal aortic pressure and keep cardiac output constant for systemic circulation. As has been demonstrated in chapter 5, this increase elicited an increase in coronary blood flow and decrease in coronary resistance. Since the bulky evidence suggested that coronary metabolic vasoregulation is closely linked to the oxygen usage by the myocardium [Feigl, 1983; Berne, 1979], one logical speculation is that the increased cardiac work induces the heart to consume more oxygen, which causes the fall of oxygen level in the intramyocardium. The myocytes may be able to detect this fall and release vasoactive substances to increase blood flow and allow the intramyocardial oxygen to recover. This theory is a "close-loop" controlling mechanism and was supported by Berne's experiments [Berne,

1979] in which they found that CBF only increased if the venous oxygen content was below a critical level of 5.5ml/100ml.

Generally the coronary sinus oxygen value represents the myocardial oxygen level<sup>1</sup>. Although the oxygen levels in the myocardium and coronary sinus are not equal, it is reasonable to assume that a fall in coronary sinus  $SO_2$  reflects a decrease in myocardial oxygen level. The coronary sinus  $SO_2$  results from the drainage volume of oxygen level in myocardium. If the close-loop controlling system operates in coronary vasodilation due to the applied intervention, a fall of  $SO_2$  (CS) initially and then recovery to the baseline would be expected during aortic constriction. However, my data, in which the coronary sinus oxygen saturation varied significantly both above and below control values during increased cardiac work while coronary resistance and coronary blood flow reacted to the change of cardiac work, did not support this hypothesis. It is more likely that harder-working myocytes released vasodilator(s) into the interstitium, regardless of the amount of oxygen in myocardium, in direct proportion to the work done by the heart, causing decreased coronary resistance and increased coronary blood flow. In most cases, the vasodilation appeared to be in excess of the  $O_2$  requirement and caused increase of the  $SO_2$ (CS). Under the modulation of neural control of coronary circulation [Feigl, 1983; Stone, 1983] during increased cardiac work,  $\alpha$ -adrenergic vasoconstriction<sup>2</sup> limited this over-reaction of metabolism to ensure adequate myocardial blood flow which caused the  $SO_2$  (CS) to remain stable or to decrease. The decrease in coronary sinus oxygen level caused by  $\alpha$ -adrenergic vasoconstriction was also found by Feigl's group [1975]. In the vagotomized anaesthetized dog, with the stimulation of sympathetic nerve heart rate, blood pressure and CBF increased, and coronary sinus

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<sup>1</sup>Refer to section 3.3.2.

<sup>2</sup>In neural control of coronary circulation,  $\alpha$ -adrenergic vasoconstriction was showed to be dominant during the increased cardiac work due to the aortic constriction. (see chapter 5)



$pO_2$  decreased from 19mmHg to 15mmHg. After  $\beta$ -adrenergic blockade, the positive inotropic and chronotropic effects due to sympathetic stimulation were blunted, but the unmasked  $\alpha$ -receptor vasoconstriction result in a fall of coronary sinus  $pO_2$  from 17mmHg to 11mmHg, which could be abolished by  $\alpha$ -receptor blockade. These data suggested that coronary sympathetic vasomotion is also involved in the regulation of myocardial oxygen tension. The results in this chapter suggest that the coronary metabolic vasoregulation functions like an "open-loop" controlling system during increased cardiac work.

During systemic hypoxia, the coronary resistance decreased in a parallel with coronary sinus oxygen saturation, whereas coronary blood flow did not exactly follow the change in  $SO_2$  (Figures 7.5 and 7.6). This suggests that the coronary vascular resistance is strongly associated with the changes of oxygen in myocardium. The lag response following the change of myocardial oxygen level invites speculation regarding the role of oxygen delivery and metabolite(s) washout in the regulation of coronary blood flow. It could be explained in two ways:

- *Direct oxygen effect on the vasodilation*

Oxygen directly acts on the vascular smooth muscle causing the relaxation<sup>1</sup>. This induces parallel changes in myocardial oxygen level and coronary resistance. While myocardial oxygen level is being led toward the normal level by increased supply of oxygen, vascular smooth muscle has a "memory" relax further ("hysteresis" phenomenon), then realizes that it is too far from the current oxygen level and over-corrects it – causing the ratio of  $cr/SO_2$  during the period of oxygen decrease to be different from that during the period of decreased oxygen supply – gradually coronary resistance is parallel with oxygen level again as shown in Figure 7.11. With

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<sup>1</sup>Refer to section 3.2.3.

hysteresis phenomenon, the more severe the change of coronary resistance due to hypoxia, the longer the lag period would be expected to be. The current data were not sufficient to clarify this.

- *Indirect oxygen effect on vasodilation*

Oxygen does not have a direct effect on the vascular smooth muscle. The lower level of myocardial oxygen causes the generation and release of vasoactive metabolite(s) from myocardium, in proportion to the degree of hypoxia, to dilate coronary vessels in order to supply enough oxygen for normal myocardial function. When the myocardial oxygen level starts to recover toward the baseline, the metabolite(s) take some time to metabolize causing the lag response. In this case, the coronary resistance should immediately follow the oxygen level in the parallel fashion after the lag period and this lag period should be related to the metabolism of the metabolite(s) responsible for the vasodilation during hypoxia. Due to the concomitant changes during systemic hypoxia, we could not determine whether coronary resistance parallels with oxygen level immediately after the lag period. The concomitant changes also complicate the study of the lag period in order to provide information about the metabolism of possible metabolite(s).

### 7.3 CONCLUSION

The metabolic control of coronary blood flow in this model is not dependent on the metabolic status of muscle expressed by coronary sinus oxygen levels. This suggests that an "open-loop" feedback system operates in coronary vasoregulation due to increased cardiac work. Coronary sinus oxygen saturation is very closely related to coronary resistance with hypoxia. This suggests either that oxygen is able to act on vascular smooth muscle directly or that low oxygen triggers a release of very short-lived vasoactive agent(s) to induce vasodilation during hypoxia.

## **CHAPTER 8**

### **RESULT 4 – DECAY OF INTRACORONARY ADENOSINE ADMINISTRATION**

## 8.1 RESULTS

### 8.1.1 Half-Life of Coronary Resistance and Coronary Flow to Exogenous Adenosine

During resting conditions, intracoronary infusion of adenosine caused no significant change in heart rate, arterial pressure and cardiac output at low doses ( $\leq 20\mu\text{g}$ ) as shown in Figure 8.1<sup>1</sup> and a significant decrease in arterial pressure at high doses ( $\geq 50\mu\text{g}$ ) as shown in Figure 8.2.

The calculated half life of mean coronary blood flow (**cqm**), coronary resistance (**cr**) and diastolic coronary blood flow (**cqd**) was independent of adenosine doses. The half lives are:

$$t_{\frac{1}{2}}(\text{cqm}) = 15.2 \pm 0.6 \text{ (seconds)}$$

$$t_{\frac{1}{2}}(\text{cr}) = 26.6 \pm 1.2 \text{ (seconds)}$$

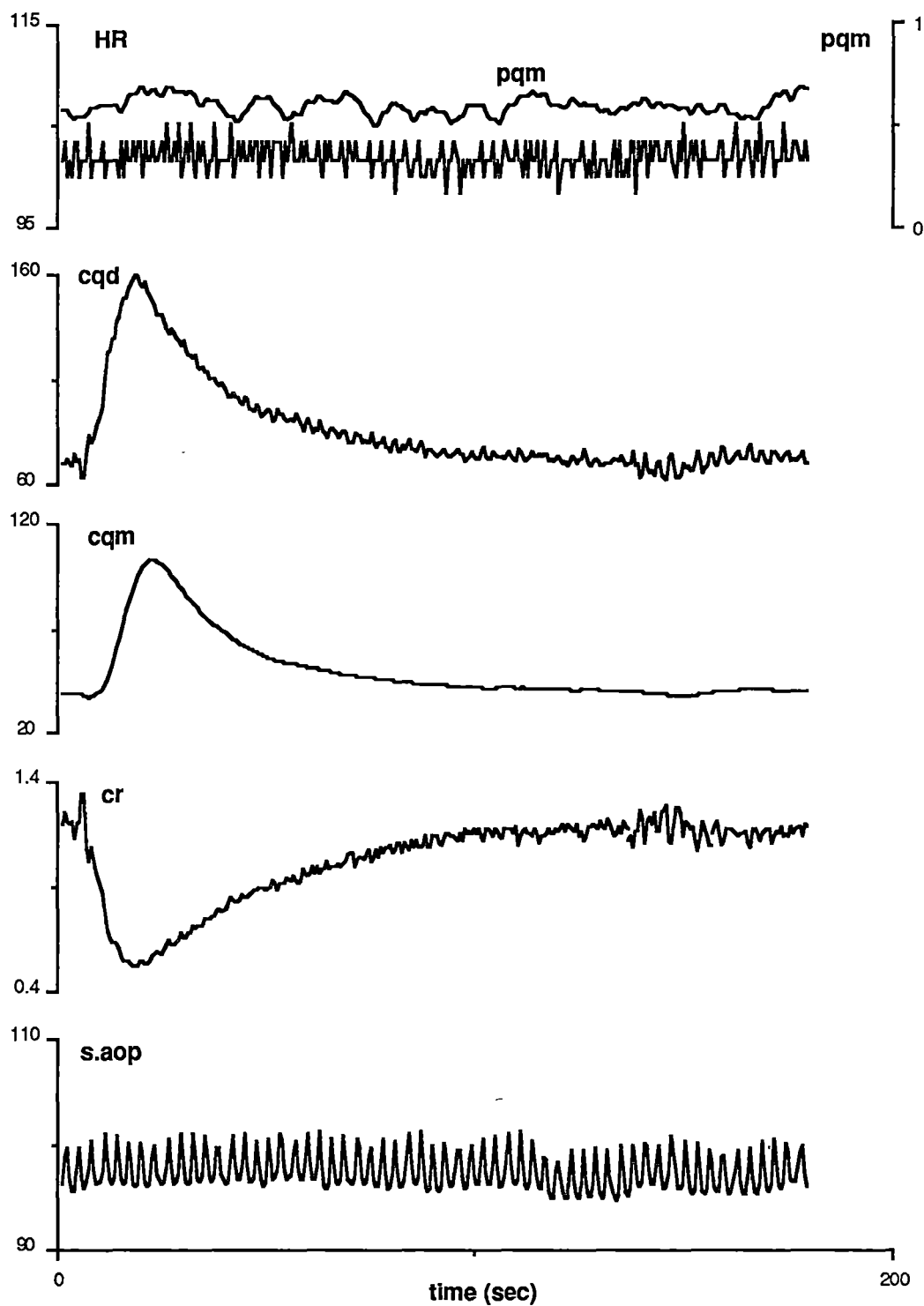
$$t_{\frac{1}{2}}(\text{cqd}) = 18.6 \pm 0.7 \text{ (seconds)}$$

### 8.1.2 Adenosine Dose - Response Curve before and after 8-phenyltheophylline

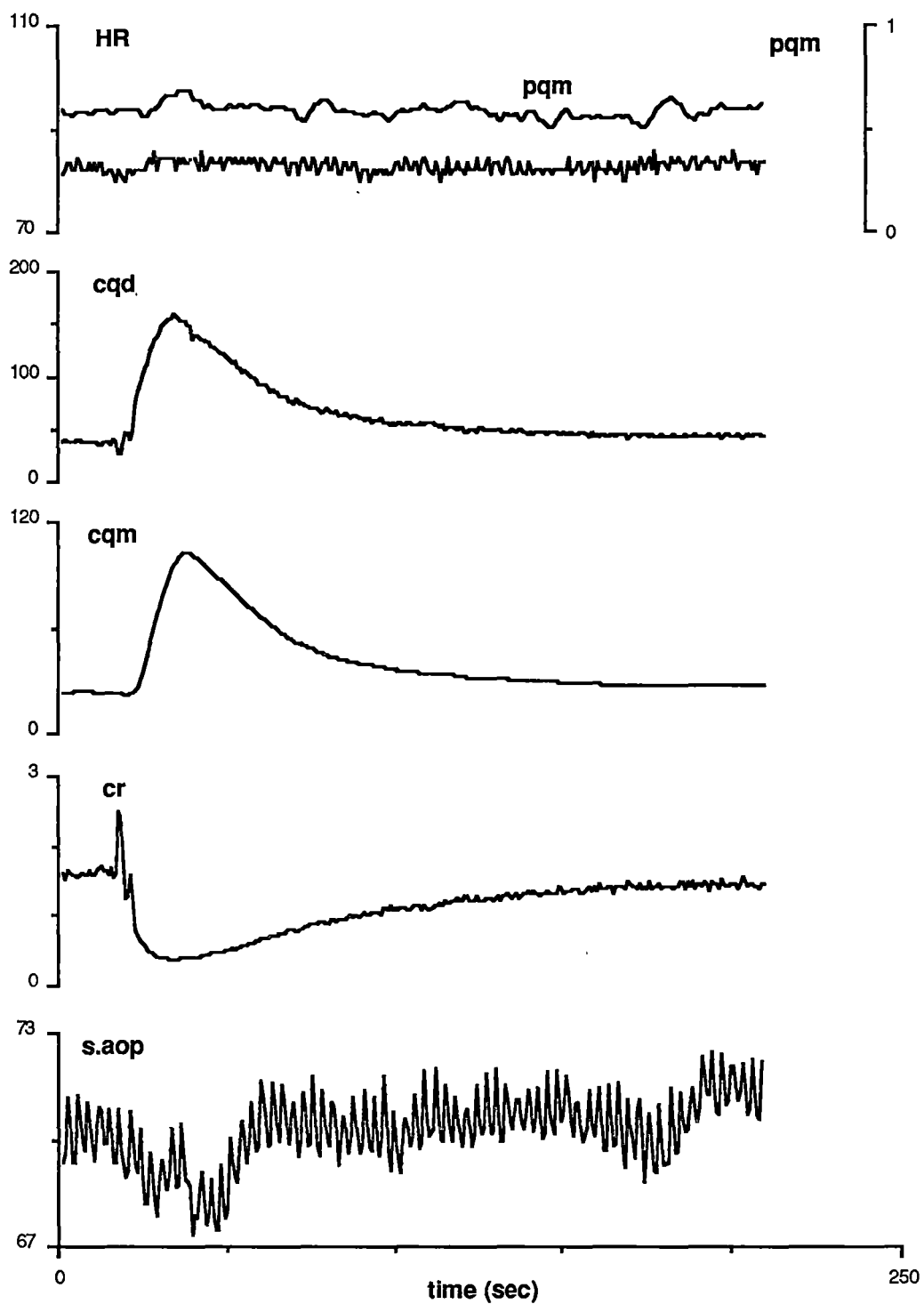
The response (expressed as the percentage change of the control) to intracoronary adenosine infusion at different doses is shown in Table 8.1 and Figure 8.4 before and after intravenous 8-phenyltheophylline (8-PT: 7.5mg/Kg) administration. Under control conditions, adenosine infusion resulted in prompt dose-related increase of coronary blood flow and decrease of coronary resistance, with a more profound response when adenosine dose was below 20 $\mu\text{g}$ .

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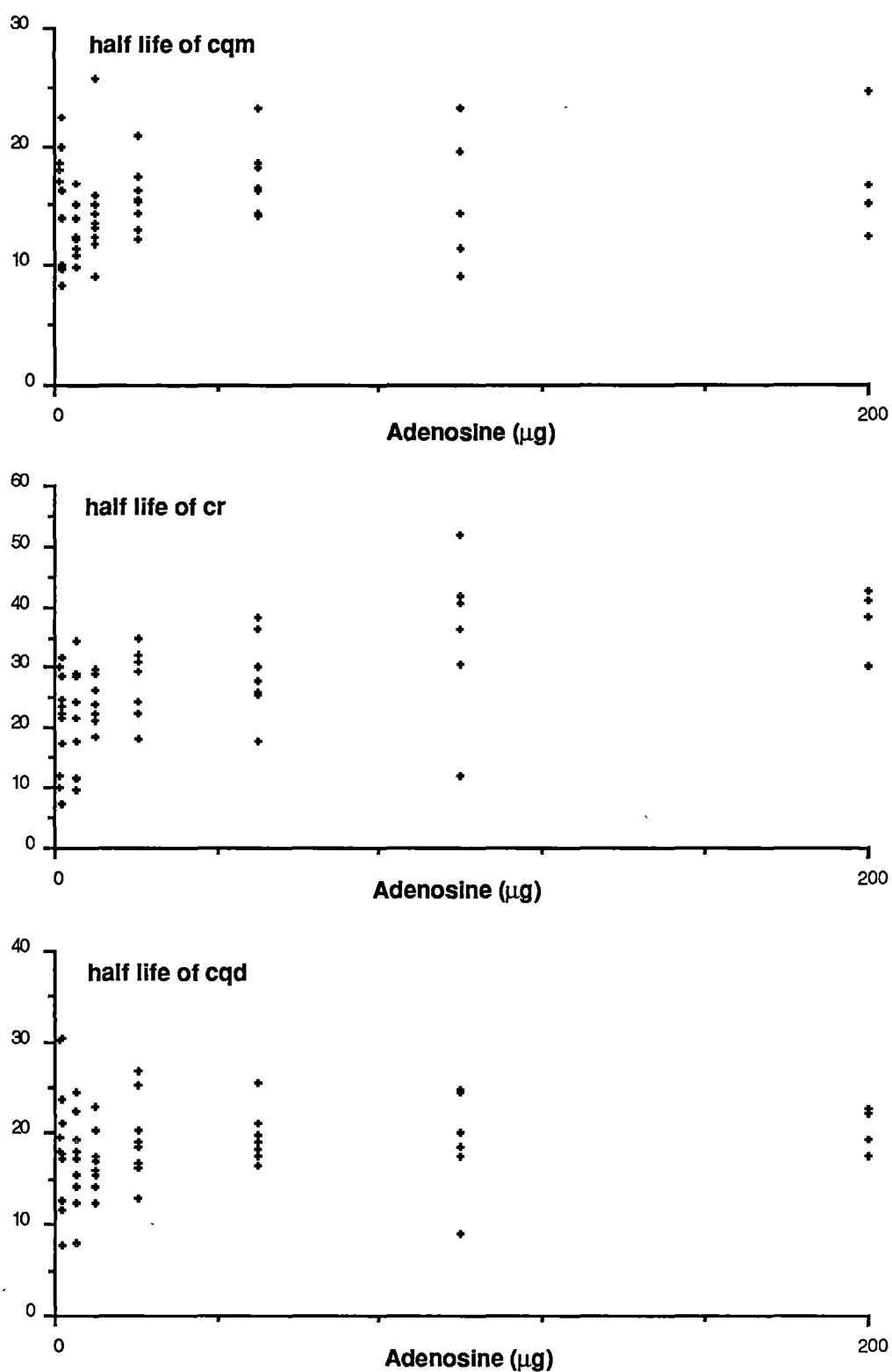
<sup>1</sup>The abbreviations are listed in glossary.



**Fig. 8.1:** The changes of hemodynamic variables to low doses of intracoronary adenosine infusion. (adenosine =  $10\mu\text{g}/0.25\text{ml}$ )



**Fig. 8.2:** The changes of hemodynamic variables to high doses of intracoronary adenosine infusion. (adenosine = 100 $\mu$ g/0.25ml)



**Fig. 8.3:** The decay (half lives) of **cqm**, **cr** and **cqd** in response to different doses of intracoronary adenosine infusion from 9 sheep.

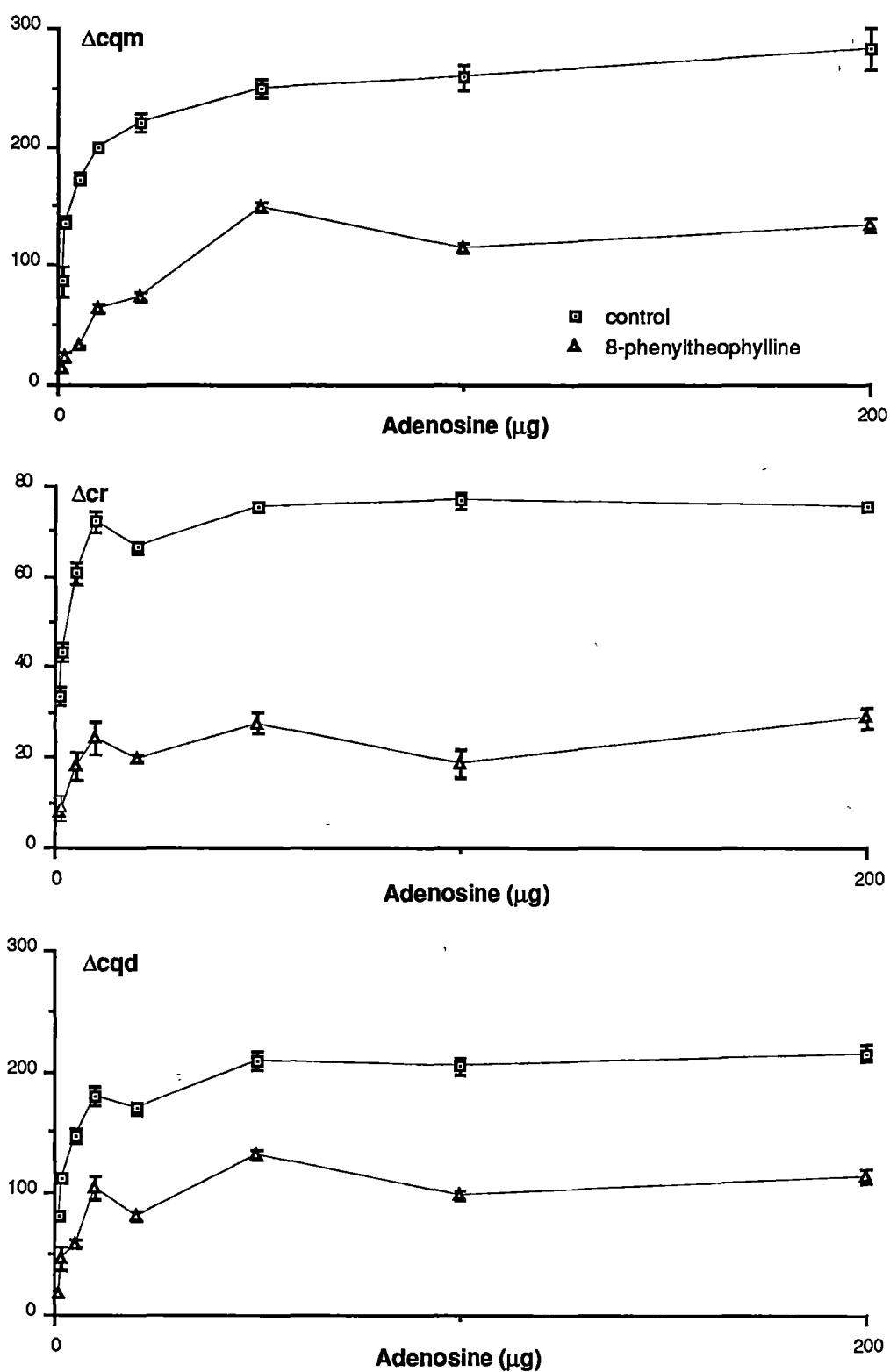


Fig. 8.4: The changes of hemodynamic variables to low doses of intracoronary adenosine infusion. (adenosine = 10μg/0.25ml)



**Table 8.1:** Response of coronary blood flow and coronary resistance to intracoronary adenosine infusion in 9 sheep

Adenosine Dose ( $\mu\text{g}$ )	No. of sample	Control			After 8-phenyltheophylline		
		$\Delta\text{cqm}$ (%)	$\Delta\text{cr}$ (-%)	$\Delta\text{cqd}$ (%)	$\Delta\text{cqm}$ (%)	$\Delta\text{cr}$ (-%)	$\Delta\text{cqd}$ (%)
1	3	86.2 $\pm$ 12.0	33.7 $\pm$ 2.1	80.4 $\pm$ 0.5	12.9*	7.69*	18.1*
2	7	136.4 $\pm$ 4.4	43.3 $\pm$ 2.0	112.3 $\pm$ 4.0	22.8 $\pm$ 4.3	8.57 $\pm$ 2.7	46.9 $\pm$ 9.3
5	9	172.8 $\pm$ 5.23	60.7 $\pm$ 2.3	146.6 $\pm$ 5.5	32.4 $\pm$ 1.5	18.0 $\pm$ 2.9	58.8 $\pm$ 2.6
10	9	199.3 $\pm$ 4.20	72.1 $\pm$ 2.3	180.1 $\pm$ 7.1	64.4 $\pm$ 4.2	24.4 $\pm$ 3.6	104.5 $\pm$ 9.3
20	8	219.9 $\pm$ 7.76	66.5 $\pm$ 1.3	169.7 $\pm$ 5.3	73.1 $\pm$ 3.5	19.7 $\pm$ 0.8	81.7 $\pm$ 3.5
50	7	250.3 $\pm$ 7.55	75.5 $\pm$ 0.5	209.1 $\pm$ 7.3	149 $\pm$ 3.6	27.5 $\pm$ 2.3	130.8 $\pm$ 5.2
100	6	258.5 $\pm$ 9.87	76.7 $\pm$ 1.7	204.6 $\pm$ 6.3	114.6 $\pm$ 3.6	18.4 $\pm$ 3.5	98.3 $\pm$ 4.1
200	4	281.94 $\pm$ 17.5	75.6 $\pm$ 0.9	215.4 $\pm$ 7.2	133.8 $\pm$ 6.0	28.6 $\pm$ 2.6	113.8 $\pm$ 5.4

\* - only two samples

**Table 8.2:** Adenosine blocking effect by 8-phenyltheophylline

variables	adenosine doses ( $\mu\text{g}$ )							
	1	2	5	10	20	50	100	200
$\Delta\text{cqm}$ (%)	84	83	81	67	66	40	55	52
$\Delta\text{cr}$ (%)	77	80	70	66	70	63	75	62
$\Delta\text{cqd}$ (%)	77	58	59	41	51	37	51	47

The blocking effect was expressed as percentage change of adenosine dose-response before and after 8-PT.

8-PT produced inhibition at all doses tested (Table 8.2; Figure 8.4). Table 8.2 shows that at lower doses (adenosine  $\leq 5\mu\text{g}$ ), 8-PT produced more significant adenosine inhibition.

## 8.2 DISCUSSION

Previously it has been reported (see section 3.3.2.4 - adenosine inhibitors) that 8-phenyltheophylline is the most potent currently available adenosine blocker having the lowest cardiac effect produced by calcium-dependent phosphodiesterase inhibition. This blocking ability was confirmed in the present study, especially at low doses of intracoronary adenosine infusion. 8-phenyltheophylline produced more than 80%

inhibition of the excess coronary flow and decreased coronary resistance in response to 5  $\mu$ g or less of adenosine. With this dose adenosine caused more changes (increase in coronary blood flow and decrease in coronary resistance) than the response caused by increased cardiac work described in chapter 5 and by systemic hypoxia as in chapter 6. Therefore, it seems reasonable to use 8-phenyltheophylline as the adenosine inhibitor to study the role adenosine plays in coronary vasoregulation due to aortic constriction and systemic hypoxia. The interesting result (Figure 8.4) was that after adenosine blockade, more adenosine (exceeding 20  $\mu$ g) did not produce more change in the coronary blood flow and coronary resistance as one might expect with competitive inhibition.

The half lives for the response to intracoronary adenosine were much longer than the response to aortic constriction observed in chapter 5. This does not support adenosine as playing a role in this vasodilation due to aortic constriction. However, if exogenous adenosine and endogenous adenosine have a different effect on vascular smooth muscle, *eg* they might combine with differential receptors on different sides of vessels, an endogenous adenosine effect on vasodilation due to increased cardiac work can not be excluded. It is likely, with the known high mobility of adenosine, that adenosine is able to interact with all receptors. Further work is needed to clarify this exception.

### 8.3 CONCLUSION

The half life of intracoronary adenosine is  $26.6 \pm 1.2$  seconds. It is much longer than the response caused by aortic constriction observed in chapter 5. It is unlikely that adenosine plays a role in the coronary vasodilation during increased cardiac work.

## **CHAPTER 9**

# **CONCLUSIONS AND RECOMMENDATIONS**

## 9.1 CONCLUSIONS

The contributions of this investigation to understanding the local metabolic vasoregulation in response to the changes of cardiac work and oxygen supply have already been discussed in each separate study of this thesis, but will be reiterated here:

- The constant and short decay of vasodilating response in response to the different degrees and durations of increased cardiac work is independent of  $\alpha$ -,  $\beta$ -adrenergic receptor inhibitions. *This suggests that common mechanisms exist in this vasoregulation, that the vasoactive metabolite(s) is very likely to be a single agent, and that the release of this vasodilator is not controlled by the neurogenic stimulation.*
- The decay of exogenous adenosine (intracoronary administration) is about 26seconds, which is much longer than that of the vasoactive metabolite. *This suggests that this vasodilator responsible for vasoregulation during increased cardiac work is not adenosine.*
- Coronary oxygen saturation – an index of the change of myocardial oxygen level – varied significantly both above and below control value (most of case showed an increase of the oxygen saturation) during increased cardiac work while coronary resistance and coronary blood flow reacted to the change of cardiac work. *This suggests that this metabolic vasodilation is in excess of the oxygen requirement, that the release of the vasodilator is related to the work done by the myocytes, not by the temporary oxygen deficiency of myocardium, and that the coronary metabolic vasoregulation functions like an "open-loop" controlling system during increased cardiac work.*
- Coronary  $\alpha$ -adrenergic vasoconstriction appears to be dominant in the neurogenic control of coronary system in response to increased cardiac work. *This suggests that the neural control system acts like a "modulator" to adjust the metabolic vasodilation and to ensure adequate blood supply to the heart, although this modulation is not perfect even for the "healthy" individual.*
- Good correlation is found between coronary resistance and oxygen level in both artery and vein during systemic hypoxia. After using adenosine

inhibitor, the correlation is improved and the change of oxygen supply level causes more change in coronary resistance and coronary blood flow. *This suggests that the metabolic vasoregulation during hypoxia is strongly related to the oxygen level and that adenosine is unlikely to act as a transmitter between myocardial hypoxia and coronary vasodilation.*

- There is a "lag" reaction of coronary blood flow and coronary resistance in response to the myocardial oxygen level, although the change of coronary flow and resistance follow very closely to the change of coronary sinus oxygen saturation. *This suggests that either oxygen has a direct effect on vascular smooth muscle or the change of oxygen level causes the production of vasodilator and this lag period is related to the metabolism of this vasodilator.*

To examine the hypothesis that **EDRF** was involved in the coronary vasodilating mechanism<sup>1</sup>, we attempted to use **NOLA** (nitro-L-arginine) to block **EDRF**. The outcome of these experiments was a prolonged hypertensive response to **NOLA** with no apparent recovery within the time of an acute experiment. In addition we found that acetylcholine produced a vasodilation after **NOLA** that could be distinguished from the normal response only by a slightly shorter duration. In conscious instrumented sheep, an intravenous bolus of 40mg/Kg **NOLA** resulted in a marked increase in systolic blood pressure which persisted for at least one hour but had returned to control by 24 hours. The blood pressure response to **NOLA** did not return to the initial response until an interval of 8 days had elapsed between repeated boluses. Thereafter, the effect of **NOLA** on the coronary artery blood flow response to increased heart work by a proximal aortic band was investigated in the instrumented open-chested anaesthetized sheep. We found, contrary to expectation, that **NOLA** produced a greater fall in coronary resistance in relation to pressure in the aorta that occurred without **NOLA**. **NOLA** appears to be a complicated compound. If **NOLA** does block **EDRF** *in vivo* (same as *in vitro*), one possible explanation for this finding

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<sup>1</sup>This was not part of this thesis.

would be that **EDRF** is acting as an endogenous free radical scavenger for some other free radical species that mediates the increased coronary blood flow during increased heart work and therefore inactivates this radical [Vial, 1991].

## 9.2 RECOMMENDATIONS

Based on the current findings of this thesis, several possibilities arise for future work:

- Almost all anaesthetics are vasoactive. The study of decay of coronary resistance in response to increased cardiac work need to be repeated in other animal models and in conscious models under full neurogenic blockade.
- The change of myocardial oxygen level in response to increased cardiac work need to be examined in conscious sheep under  $\alpha$ -adrenergic blockade.
- The characteristics of endogenous adenosine need to be clarified – is it the same as exogenous adenosine?
- Other putative vasodilator's decay (especially **EDRF**) need to be studied in order to search the final agent responsible for vasoregulation during increased cardiac work.
- Using a coronary perfusion model (which allows coronary perfusing pressure be controlled and some metabolic parameters in reservoir blood be controlled in desirable level) the time course of coronary resistance in response to the different level of oxygen supply can be studied. Also under these conditions, the "lag" response<sup>1</sup> of coronary blood flow to the change of oxygen supply can be confirmed and investigated precisely.
- Further experiments with hypoxia and continuous coronary sinus oxygen measurements under various blockers are required. From my data there is

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<sup>1</sup>Refer to chapter 7.

a suggestion that the "lag" in response of coronary resistance has a similar time course to that produced by increased work.

- we have hypothesized this vasodilation could be due to a free radical (*eg* NO), but preliminary studies using NOLA are against this. Further studies using oxygen free radical scavengers such as SOD (superoxide dismutase) are required to rule out the presence of a dilating free radical based on oxygen which forms the common pathway for this vasodilating response.

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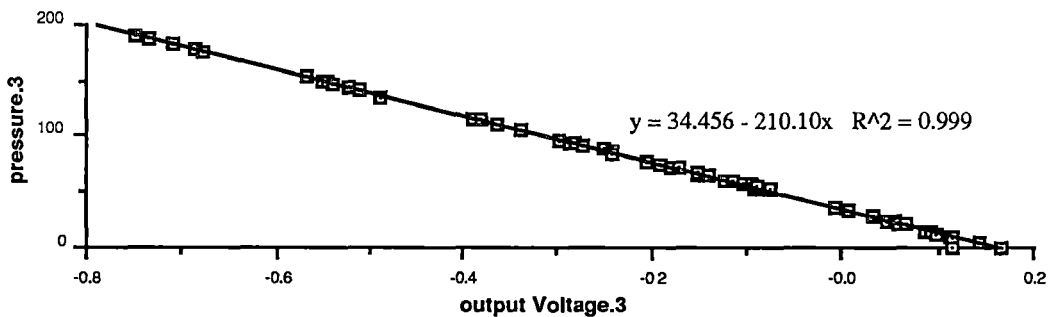
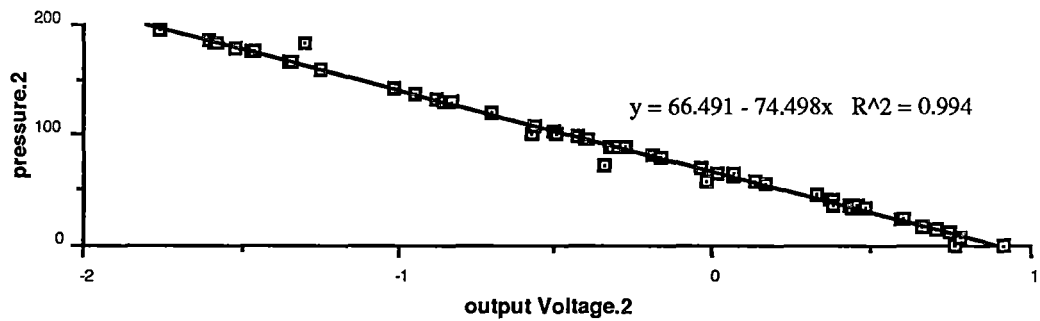
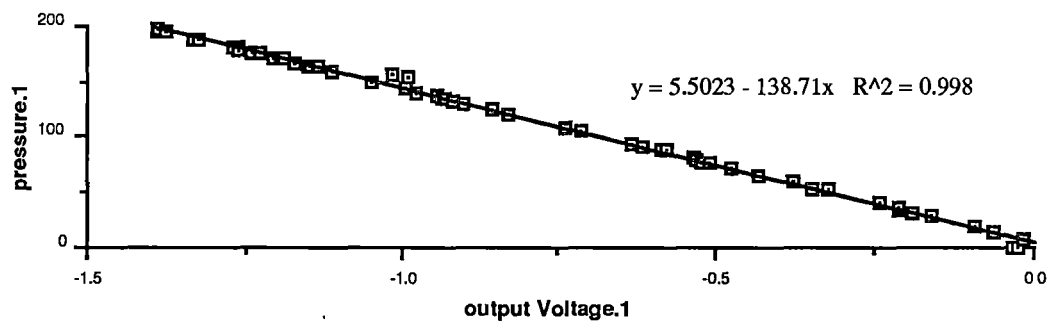
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Xhonneux R, Schaper W: The  $pO_2$  in the coronary sinus. Correlation studies with other circulatory and respiratory parameters based on a population of 500 dogs. *Prog Respir Res* 3: 89-93, 1969

## APPENDIX A

### The Calibration for Pressure Transducers

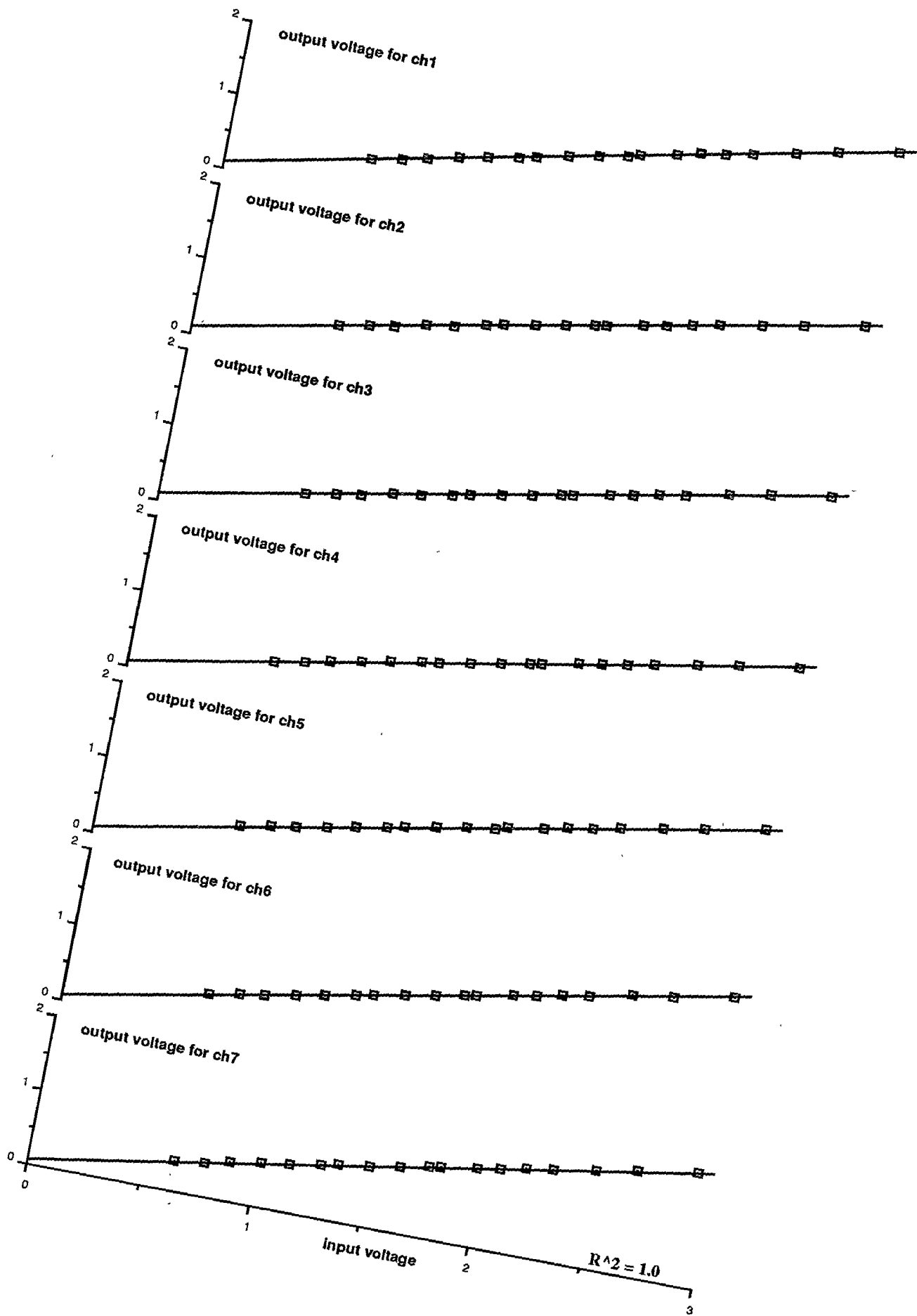
The following graphs demonstrate the pressure and output voltage relationship for three Statham P23ID pressure transducers which were used in the experimental work. In the pressure range 0 - 180mmHg, all the transducers showed a linear pressure-voltage relationship ( $R > 0.99$ ).



## **APPENDIX B**

### **Calibration for 7-channel analog tape recorder**

The following shows the input and output voltage relationship for the seven channels of the analog tape recorder. Almost perfect linearity was observed.



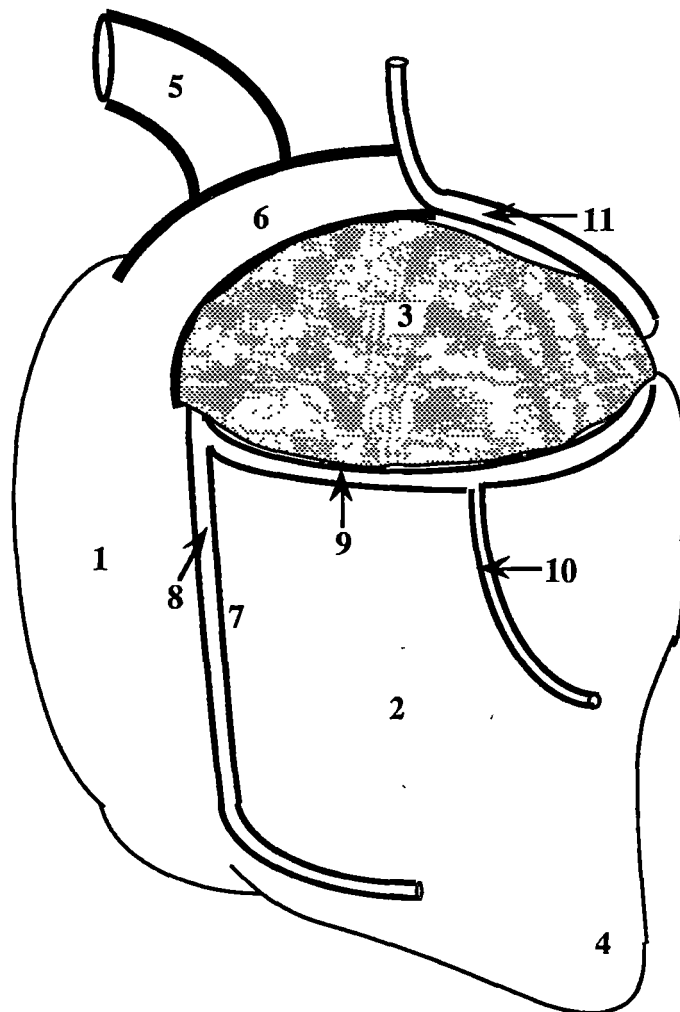
## **APPENDIX C**

### **Anatomy of the Coronary Circulation of the Sheep**

The sheep heart is located between the second and the fifth intercostal space. The blood supply to the heart is from the left and right coronary arteries. Principally, the left coronary artery supplies the left heart and the right coronary artery supplies right heart, but there is some overlap. As in Figure C.1, the left coronary artery arises at the root of the aorta behind the left aortic valve and passes back medial to the pulmonary artery. Near the origin of the left coronary artery it divides into left the anterior descendant (LAD) and the circumflex branch. The circumflex branch follows the coronary groove to near the caudal border of the heart and ends close to the termination of the right coronary artery. LAD descends the left longitudinal groove. Another descending branch leaves close to the termination of the circumflex branch and is distributed over the caudal wall of the ventricle (during my work, I discovered the location of this branch varies from sheep to sheep). The right coronary artery arises behind the right aortic valve and passes along the right part of the coronary groove and passes down the right longitudinal groove of the heart.

After passage through the capillary beds, most of the venous blood returns to the right atrium through the coronary sinus (CS). There are three major veins, the great, middle and small cardiac veins, in the coronary venous system. The great vein collects most of venous blood in the area supplied by the circumflex artery. It begins near the apex and accompanies the descending branch of the left coronary artery. At the coronary groove it turns along the left side around the caudal border of the heart where it joins with the vena hemiazygos and opens into the CS in the right atrium. The middle vein collects most of venous blood in the area supplied by the right coronary artery. It opens independently into the right atrium immediately cranial to the CS. The small veins mainly collect venous blood from the right and left atrium.

The important difference between sheep and human heart anatomy, is the **Vena Hemiazygos** system. It begins in the abdomen below the first lumbar vertebra and drains the venous blood from wall of the thorax on both left and right side. It passes through the diaphragm, dorsal to the aorta, and leaves parallel with the level of the hiatus aorticus, then turns ventrally and crosses the aorta obliquely to reach the caudal border of the origin of the pulmonary artery. Here it turns caudo-ventrally across the left atrium, medial to the left phrenic nerve, to join with the great cardiac veins. Because of its special route, we can reach the coronary sinus through the hemiazygos vein while it crosses the left atrium.



**Fig.c.1:** Diagrammatic representation of the left side of the sheep heart.  
(see text for more details)

(1. Right Ventricle; 2. Left ventricle; 3. Left Atrium; 4. Apex; 5 Aorta; 6. Pulmonary Artery; 7. Longitudinal groove; 8. Left Anterior Descendens; 9. Circumflex Artery; 10. Intermediate groove and descendens branch; 11.Hemiazygos vein ).

## APPENDIX D

### The Regression Analysis for Chapter 6

The following graphs demonstrate the relation between coronary resistance (**cr**) and other parameters (**pH**, **pCO<sub>2</sub>**, **pO<sub>2</sub>**, **SO<sub>2</sub>**) from both arterial blood (**ARTERY**) and venous blood (**VENOUS**) and myocardial oxygen consumption – **MVO<sub>2</sub>**) for hypoxic experiments in chapter six. It consisted of 8 sheep. Each sheep was analysed separately before and after adenosine (ADO) inhibition by 8-phenyltheophylline. Data expressed as:

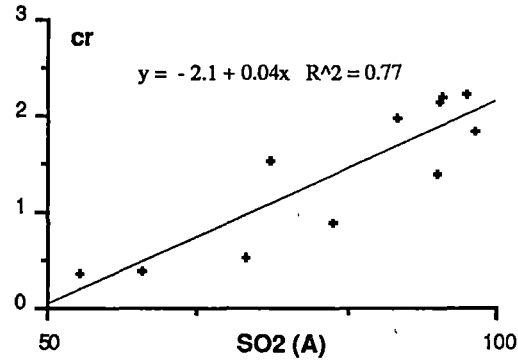
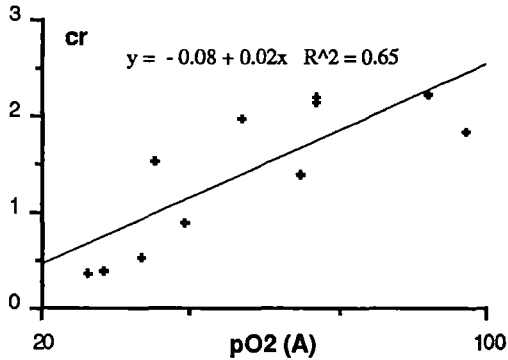
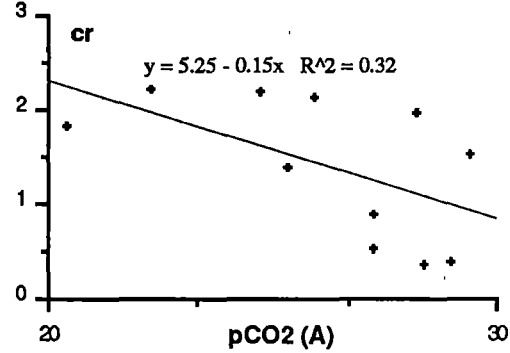
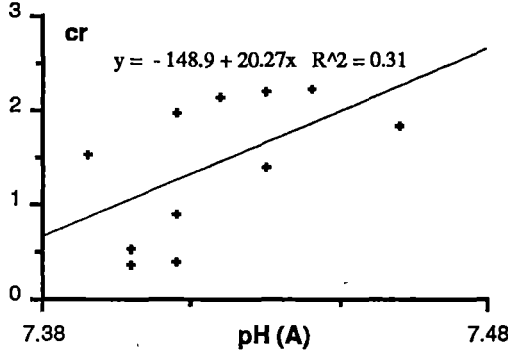
$$y = a + bx$$

where **y** represents **cr** and **x** represents the other parameter. **R<sup>2</sup>** is the *Correlation Coefficient*.

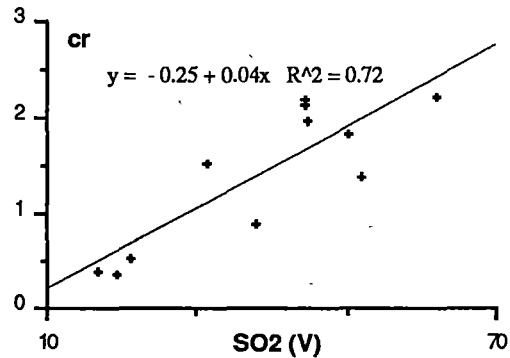
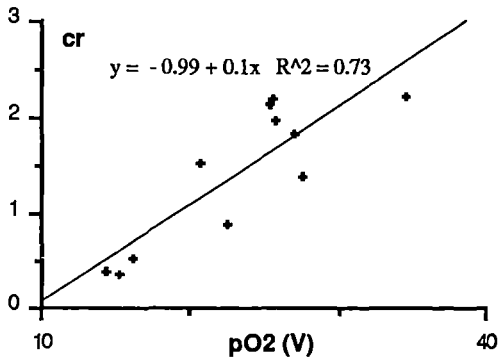
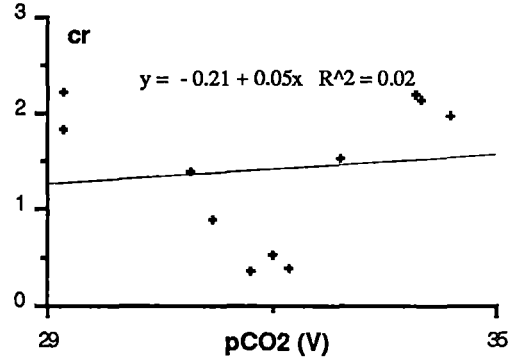
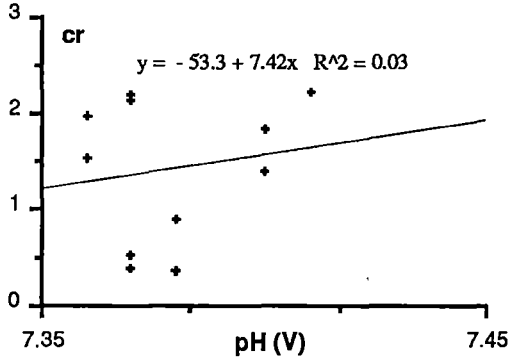


1. Data from experiment 90.10.16

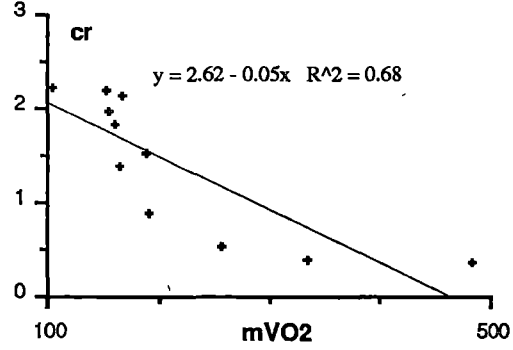
A: ARTERY (control)



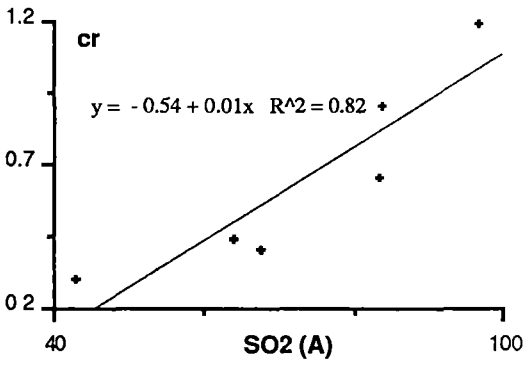
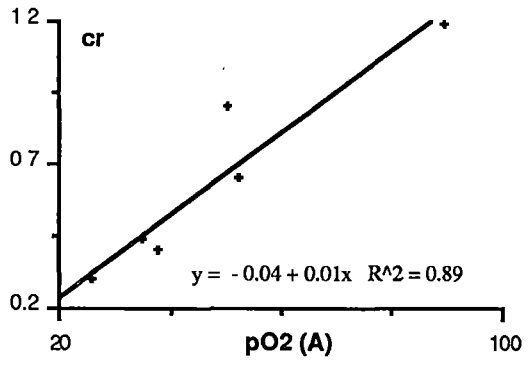
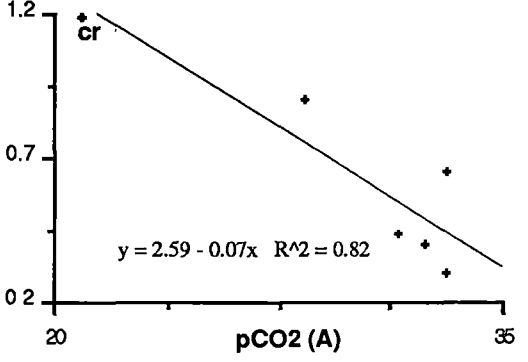
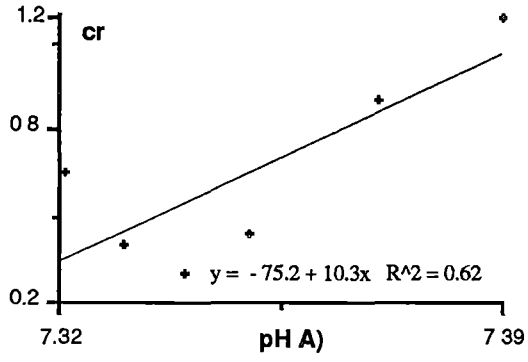
B: VENOUS (control)



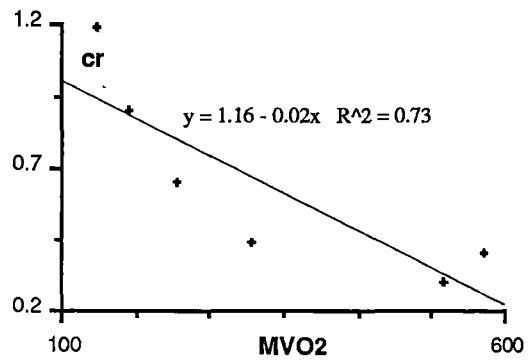
**C: MYOCARDIAL OXYGEN CONSUMPTION (control)**



**D: ARTERY (ADO inhibition)**

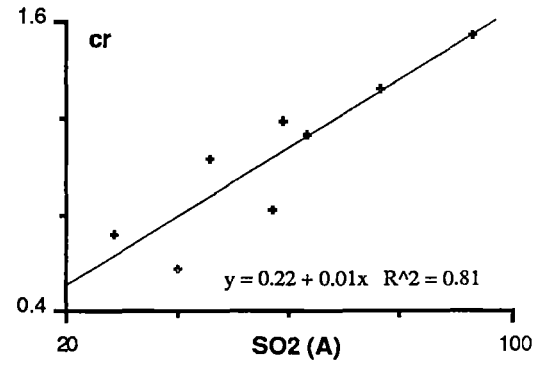
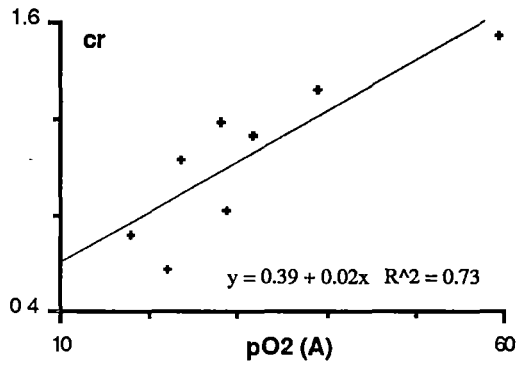
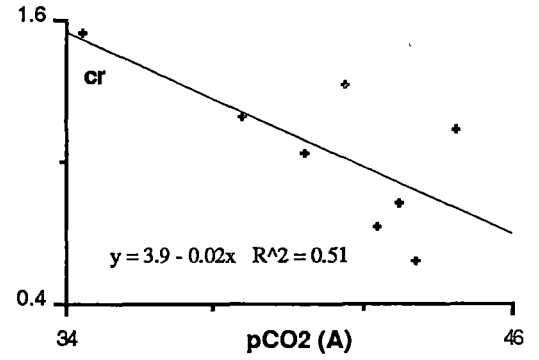
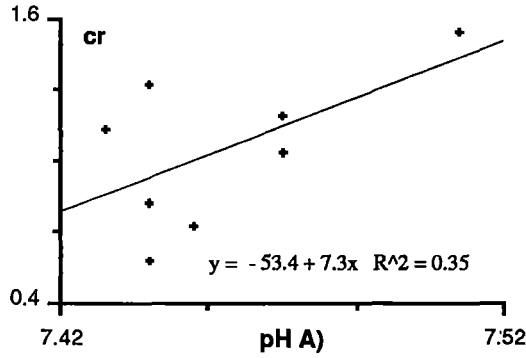


**E: MVO2 (ADO inhibition)**

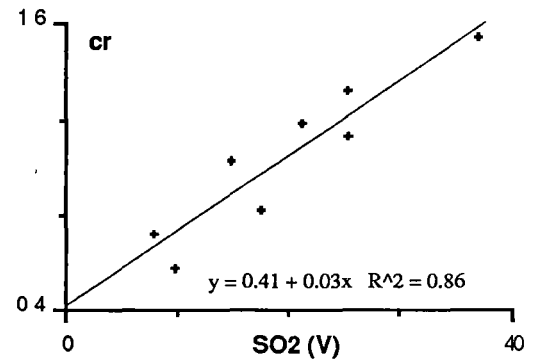
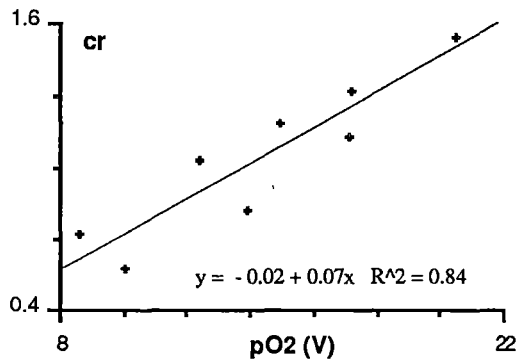
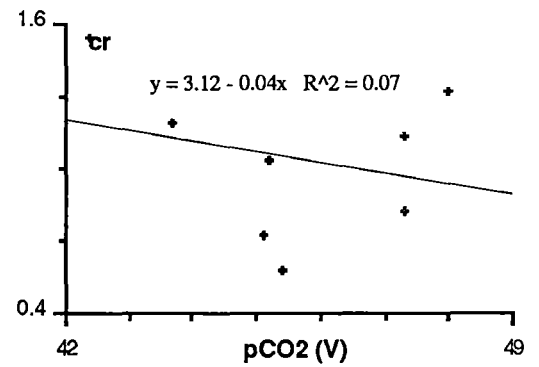
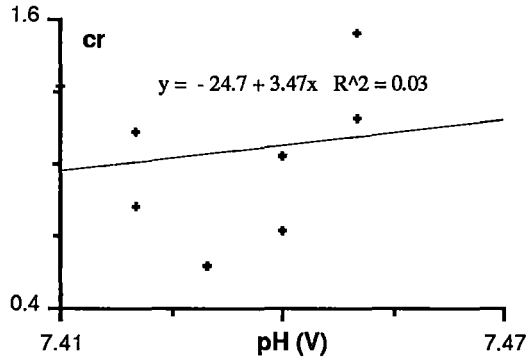


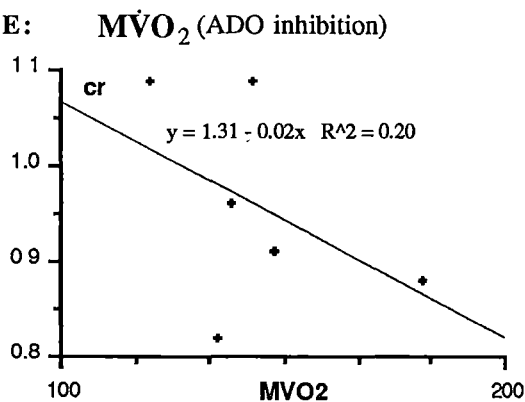
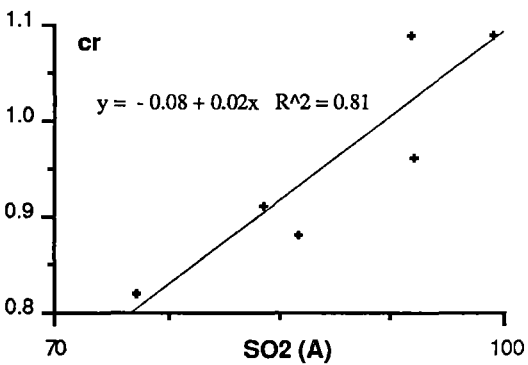
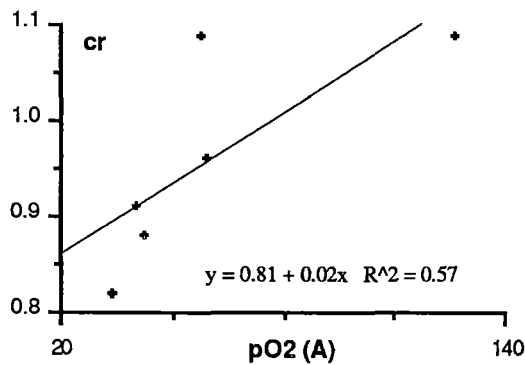
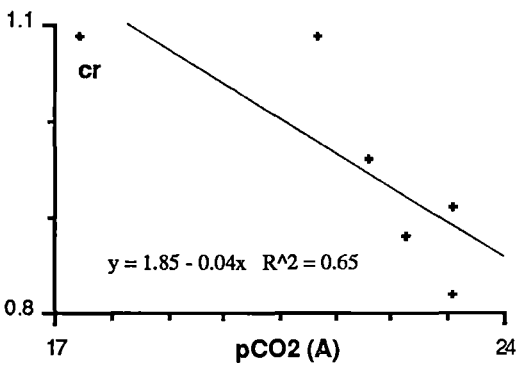
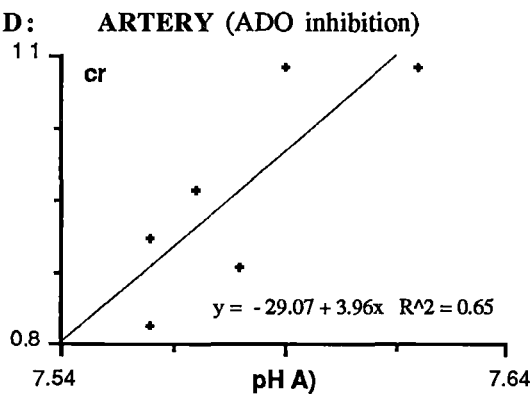
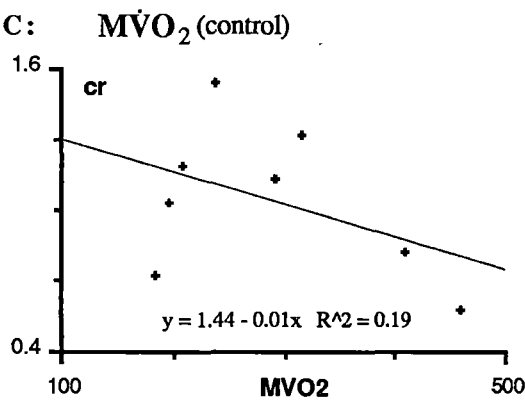
## 2. Data from experiment 90.11.16

### A: ARTERY (control)



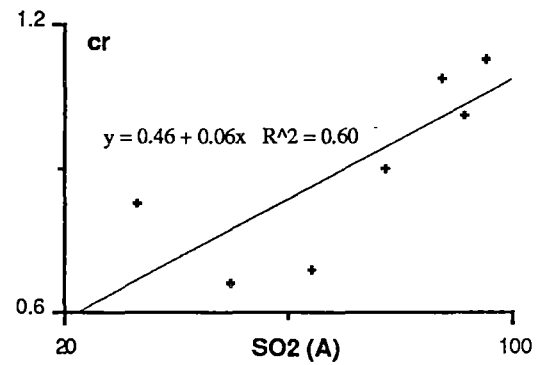
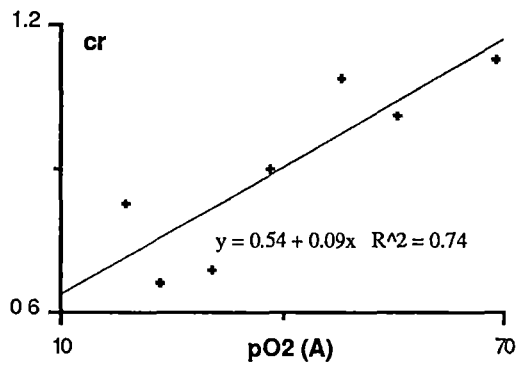
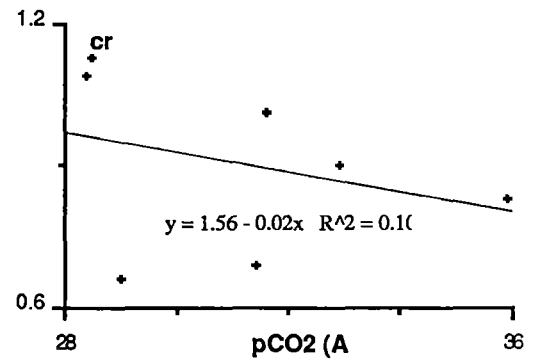
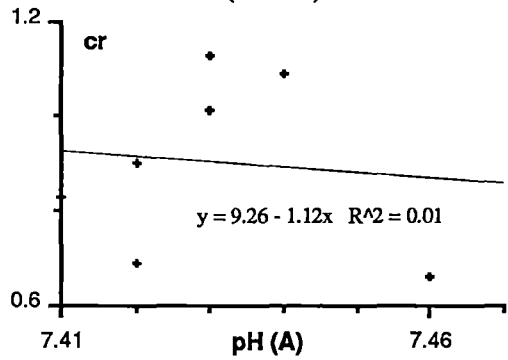
### B: VENOUS (control)



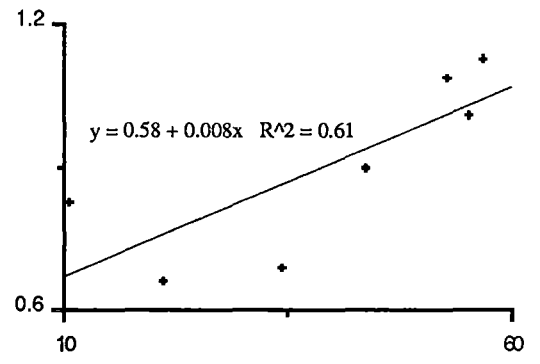
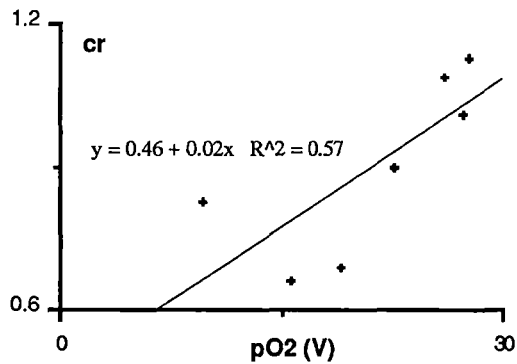
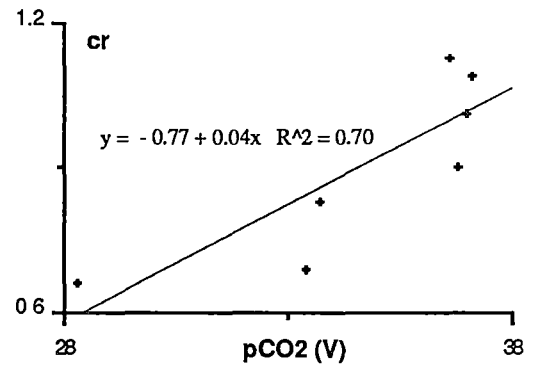
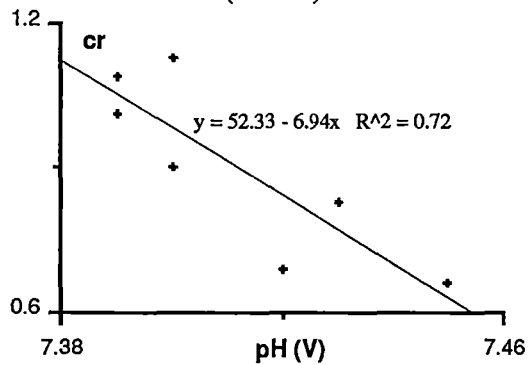


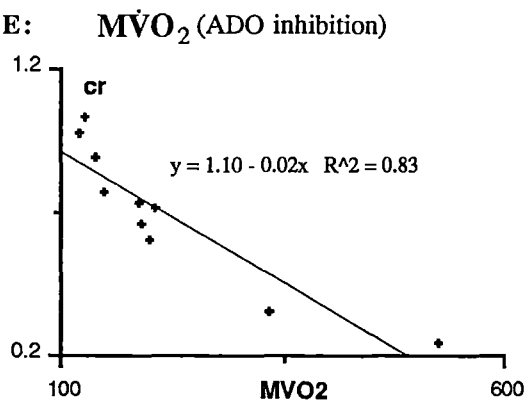
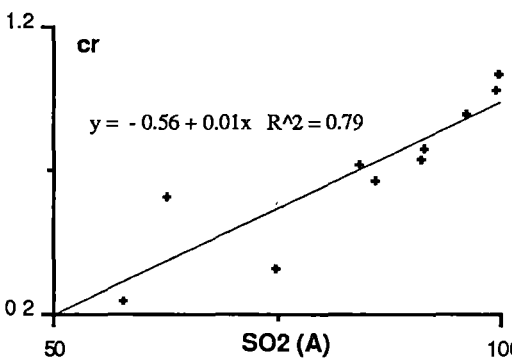
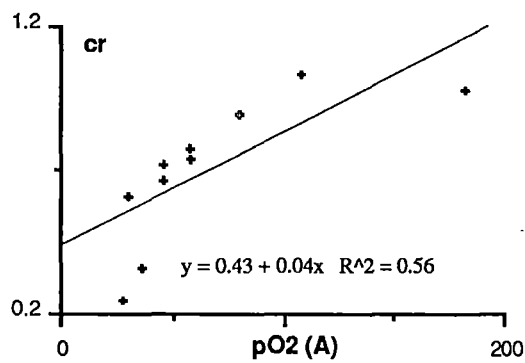
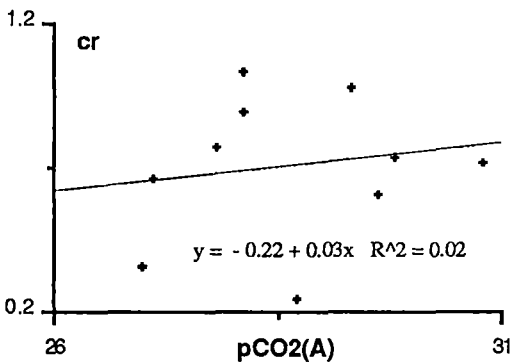
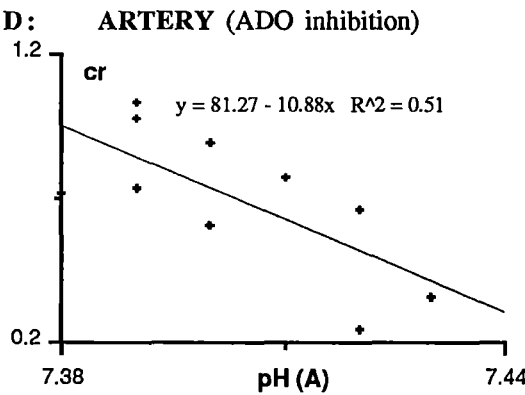
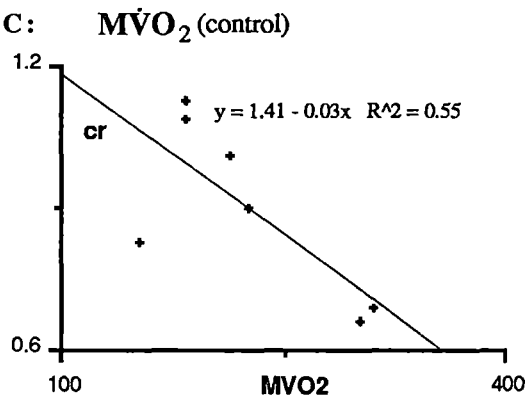
### 3. Data from experiment 90.9.25

#### A: ARTERY (control)



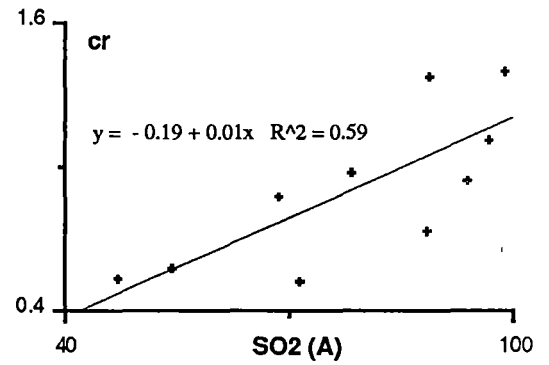
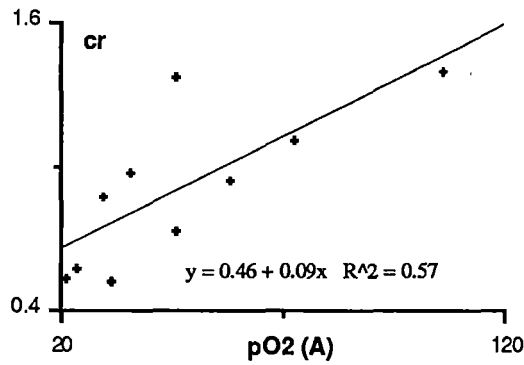
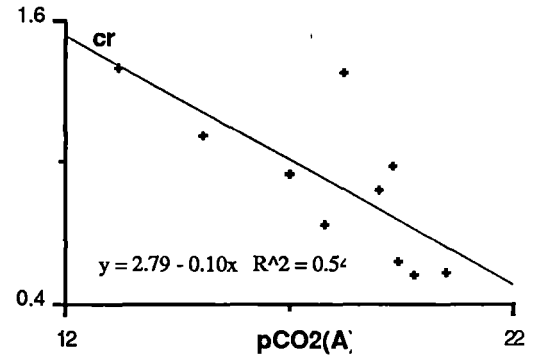
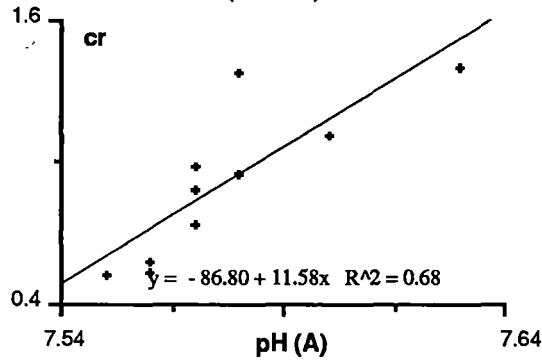
#### B: VENOUS (control)



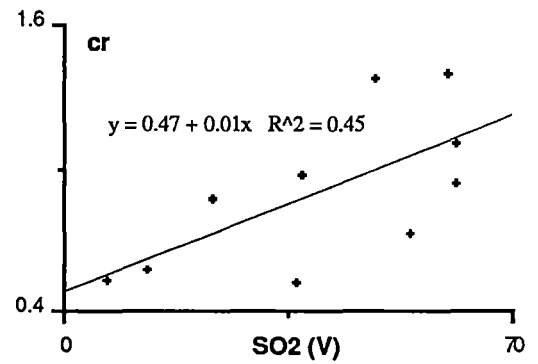
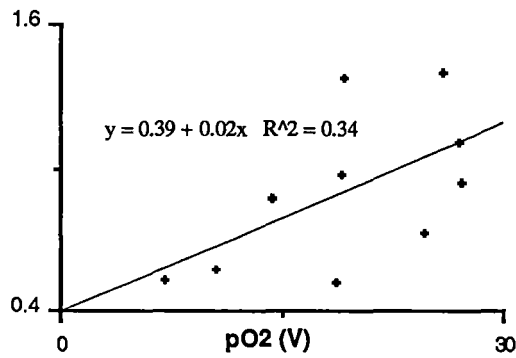
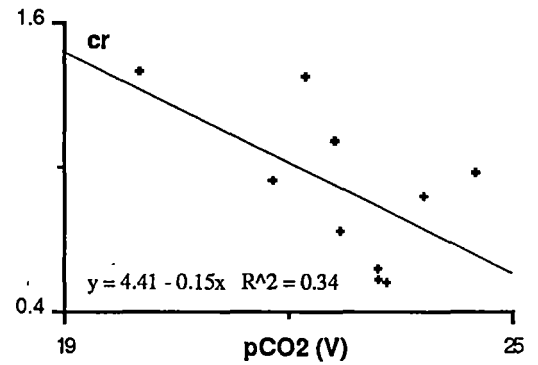
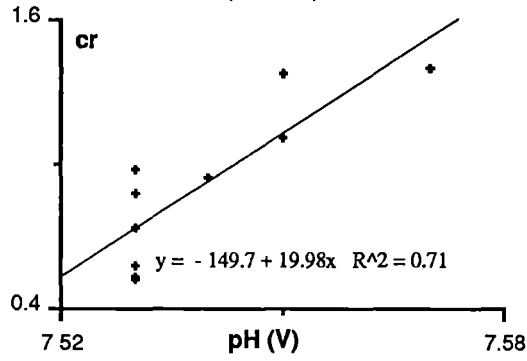


#### 4. Data from experiment 90.11.12

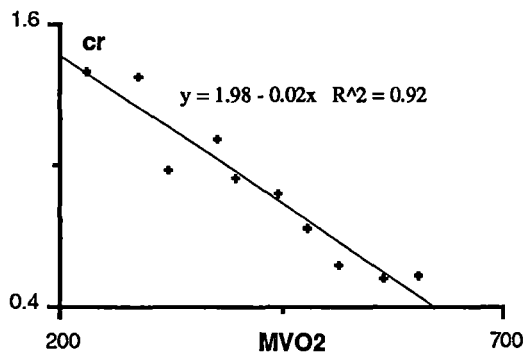
##### A: ARTERY (control)



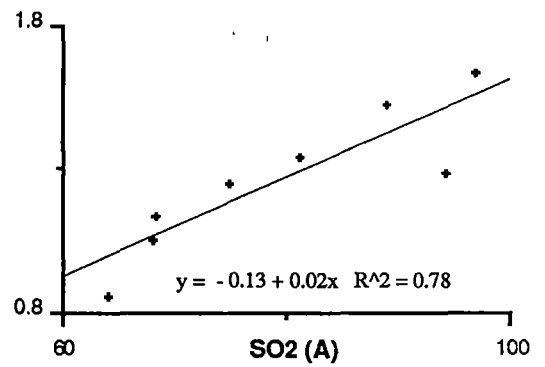
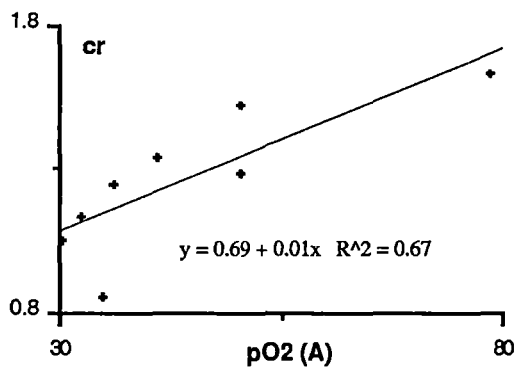
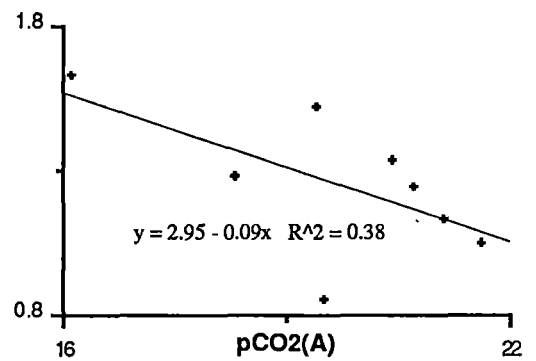
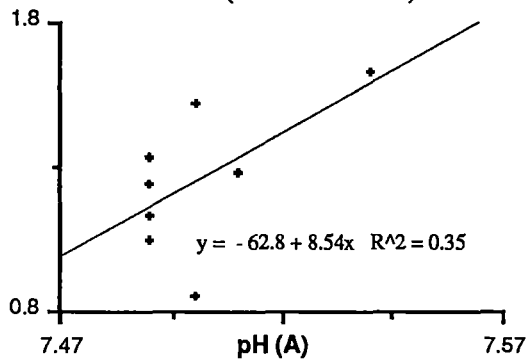
##### B: VENOUS (control)



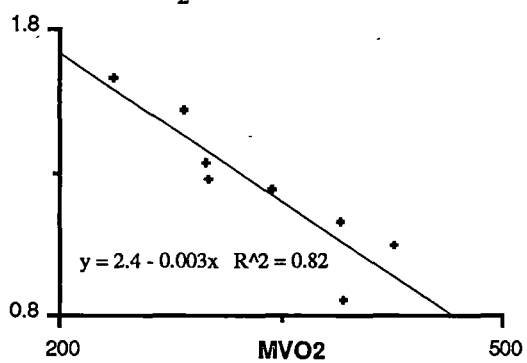
C:  $\dot{MVO}_2$  (control)



D: ARTERY (ADO inhibition)



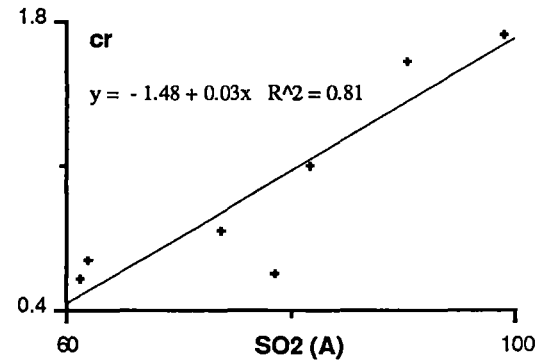
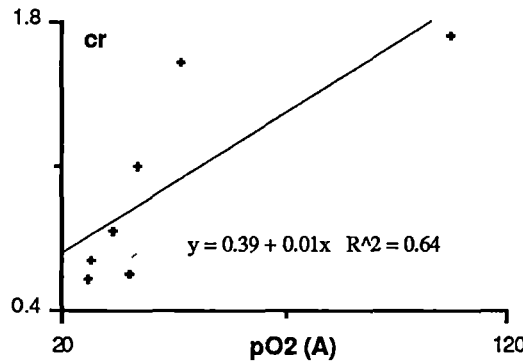
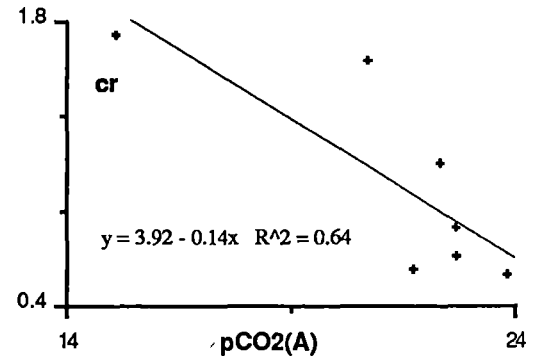
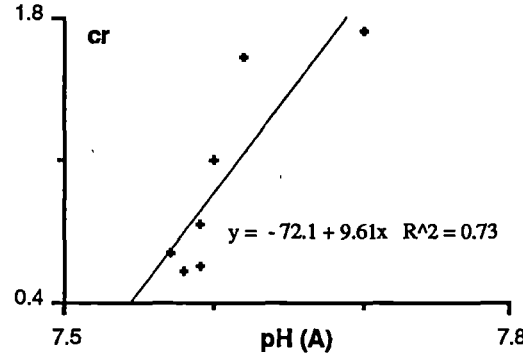
E:  $\dot{MVO}_2$  (ADO inhibition)



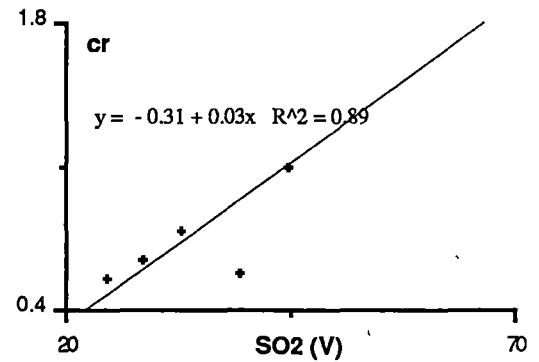
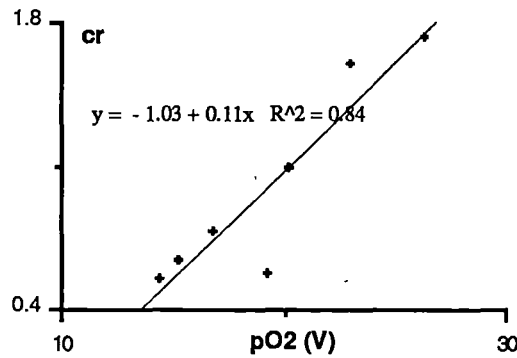
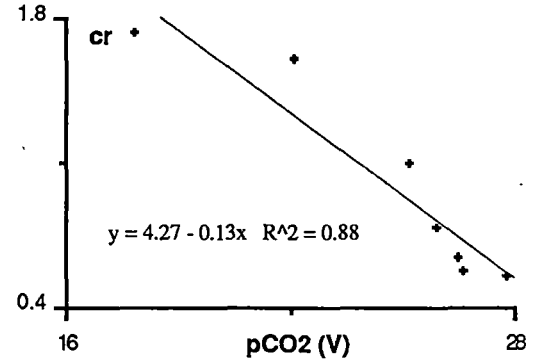
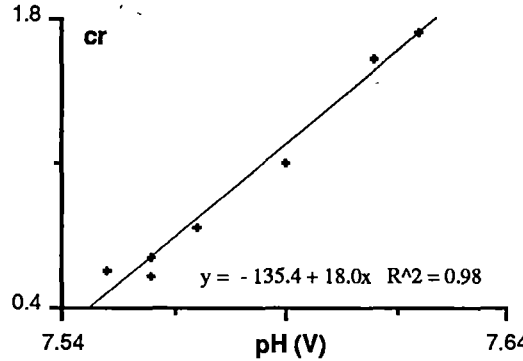


# 5. Data from experiment 90.11.20

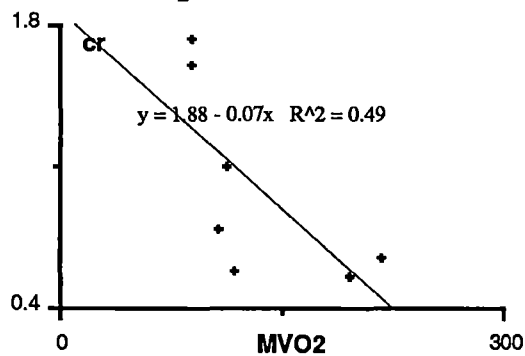
## A: ARTERY (control)



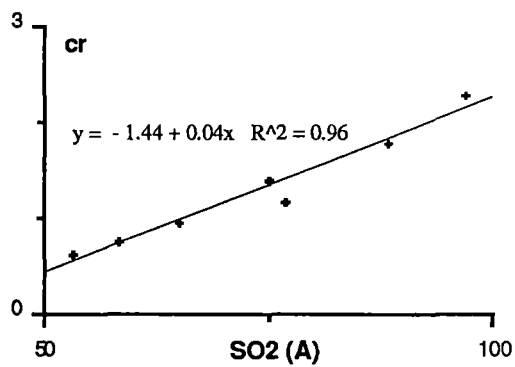
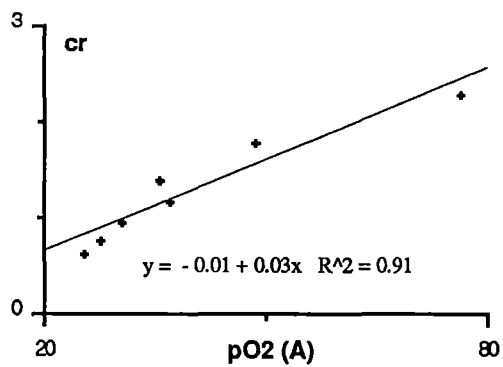
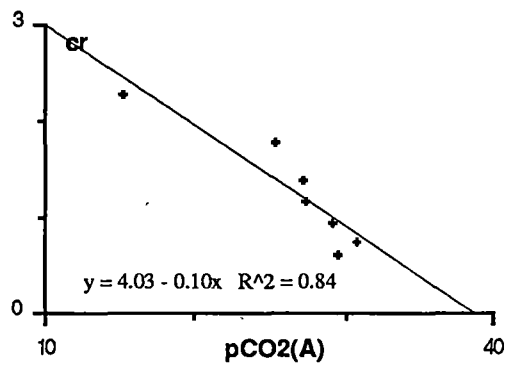
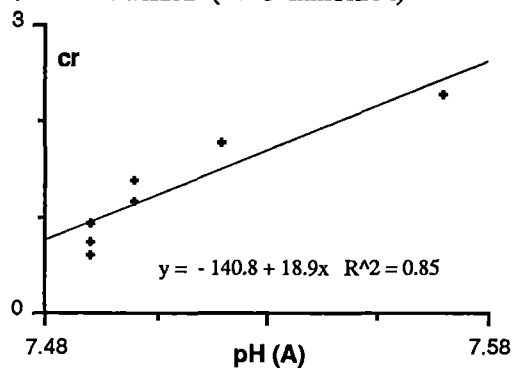
## B: VENOUS (control)



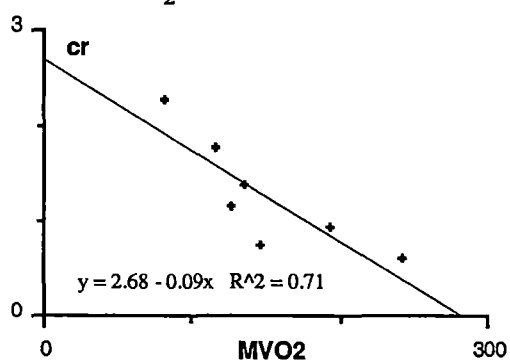
C:  $\dot{MVO}_2$  (control)



D: ARTERY (ADO inhibition)

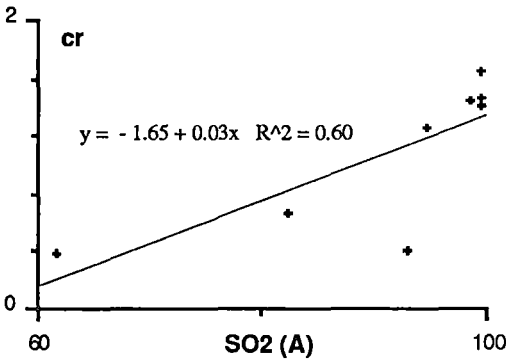
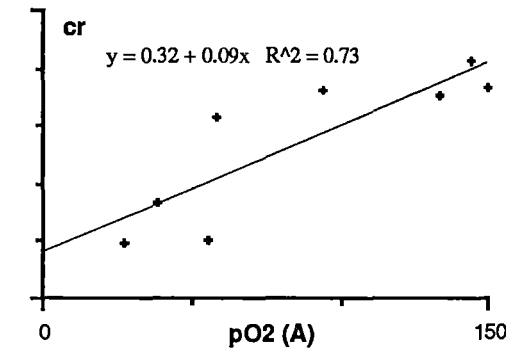
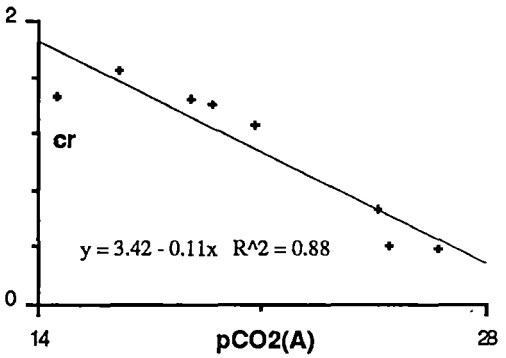
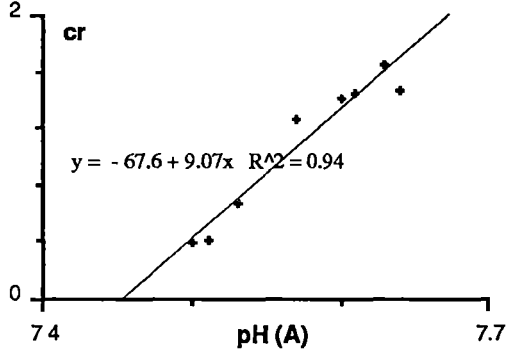


E:  $\dot{MVO}_2$  (ADO inhibition)

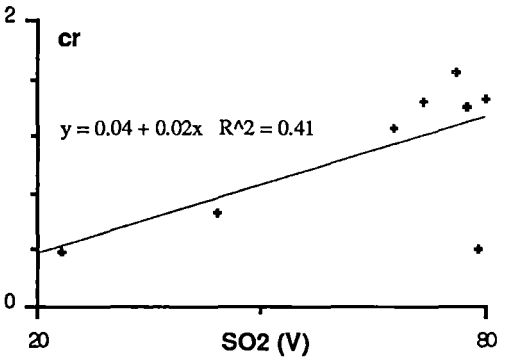
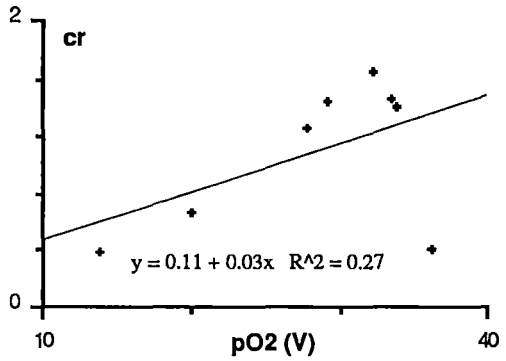
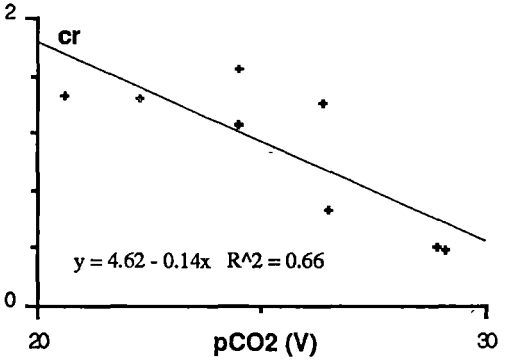
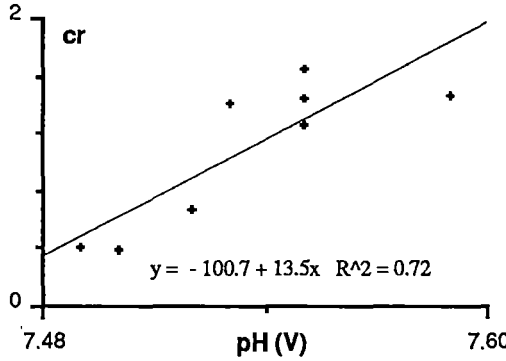


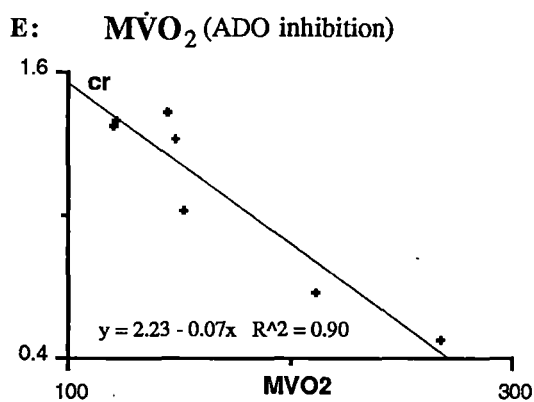
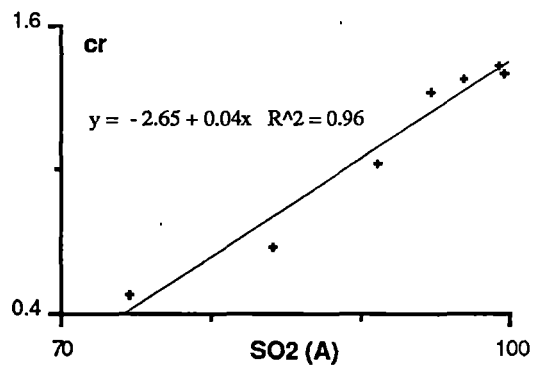
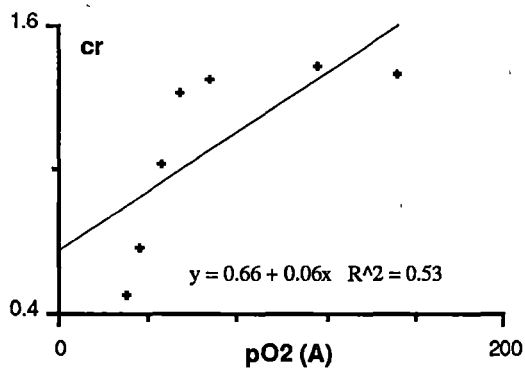
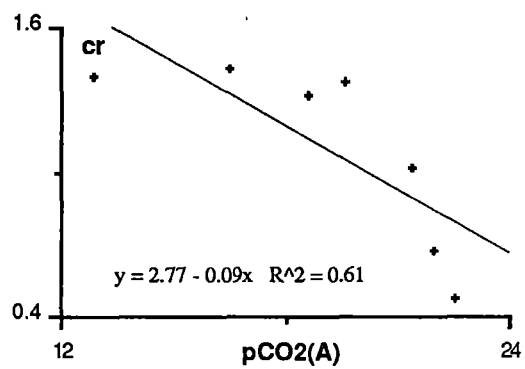
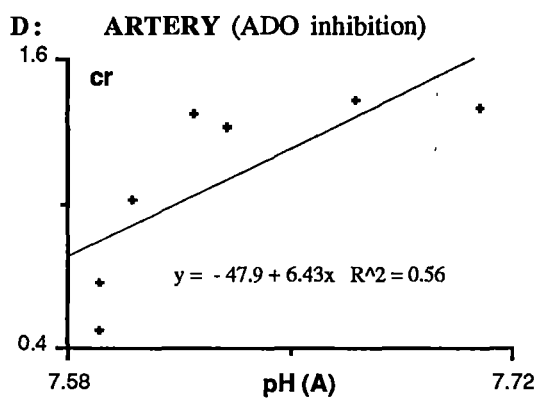
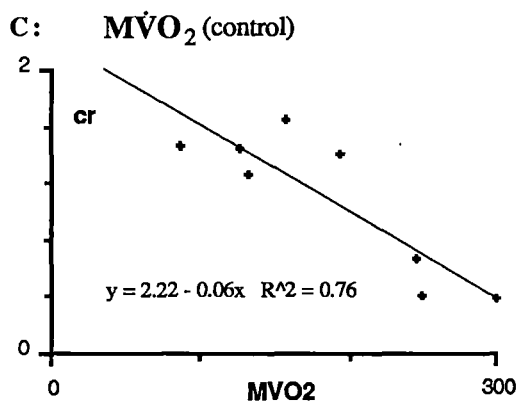
# 6. Data from experiment 90.10.9

## A: ARTERY(control)



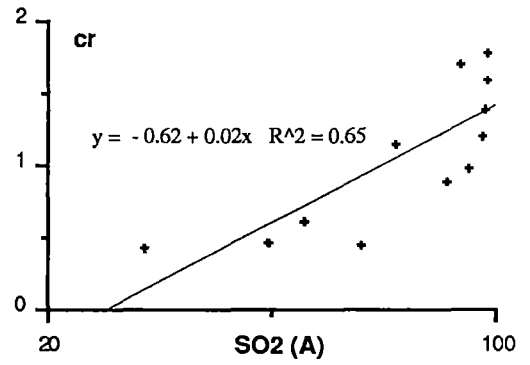
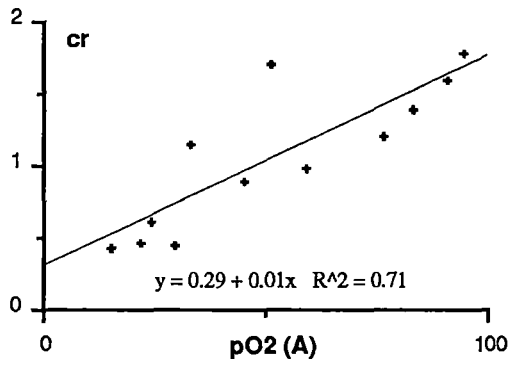
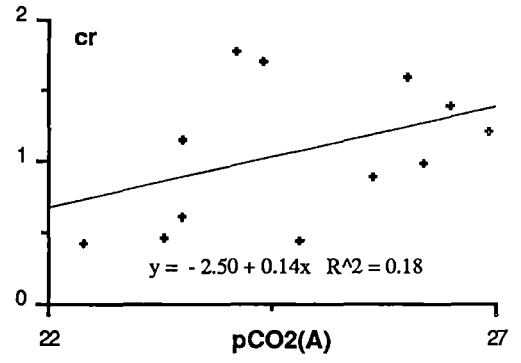
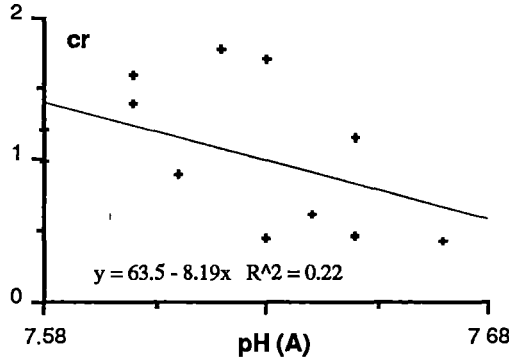
## B: VENOUS (control)



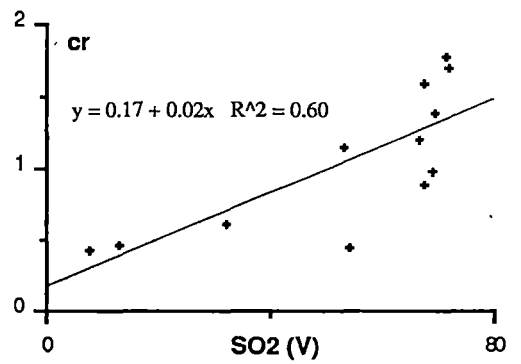
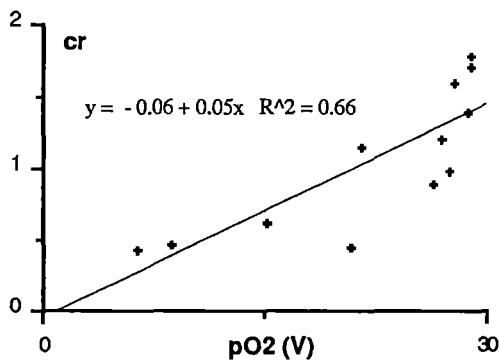
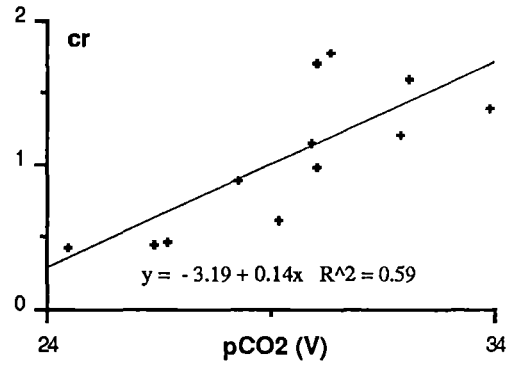
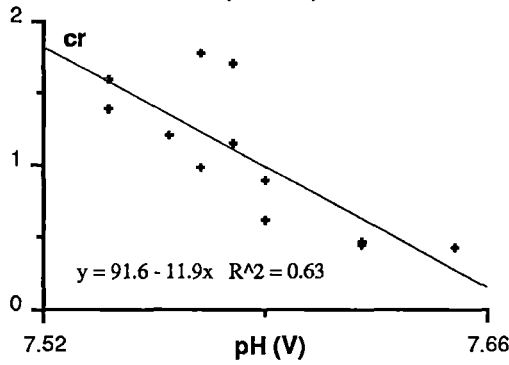


# 7. Data from experiment 90.9.24

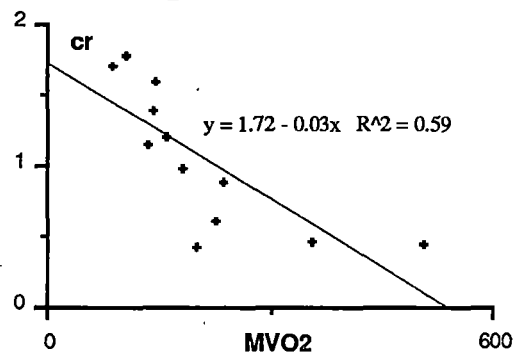
## A: ARTERY (control)



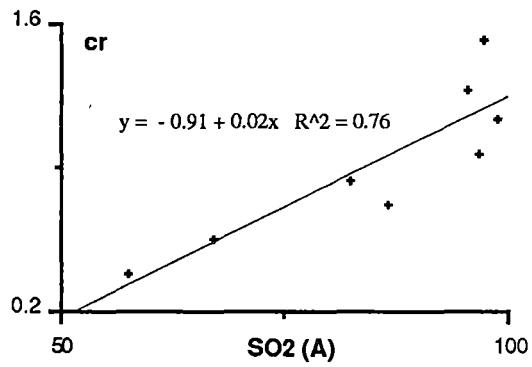
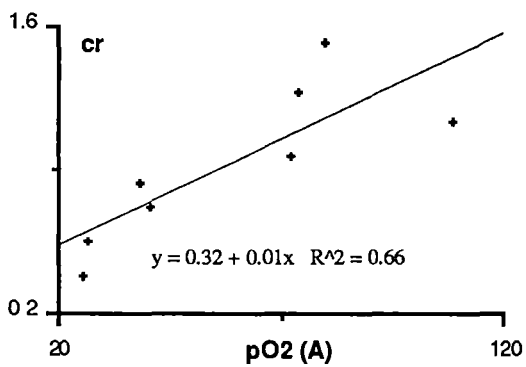
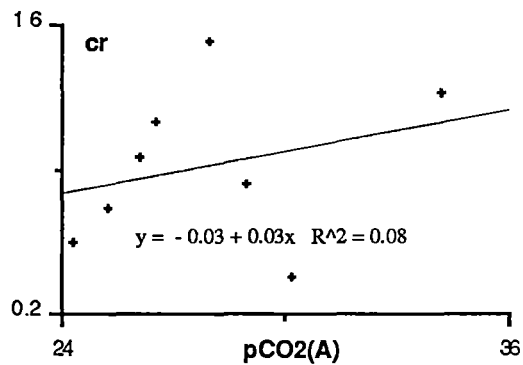
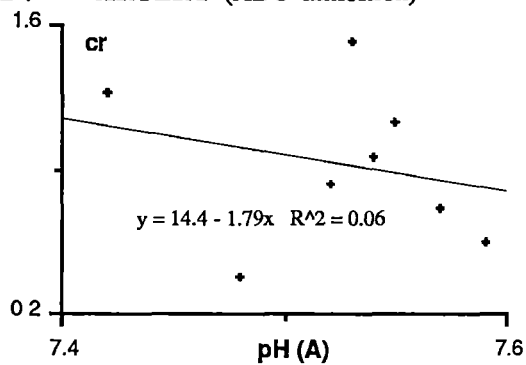
## B: VENOUS (control)



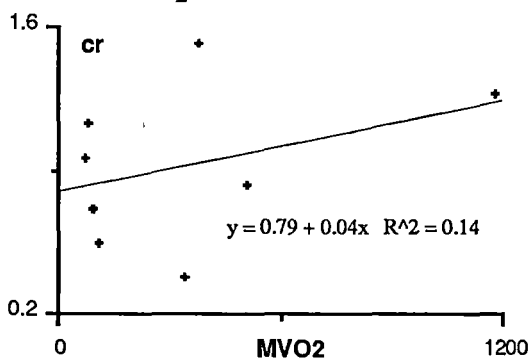
**C:  $\dot{MVO}_2$  (control)**



**D: ARTERY (ADO inhibition)**

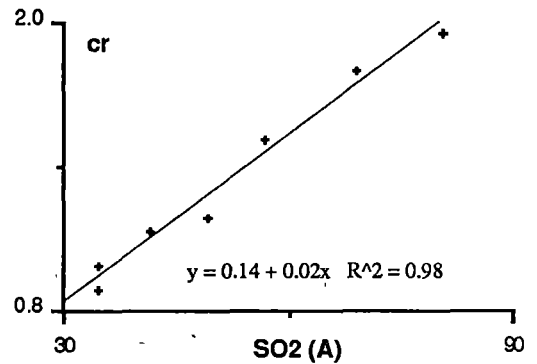
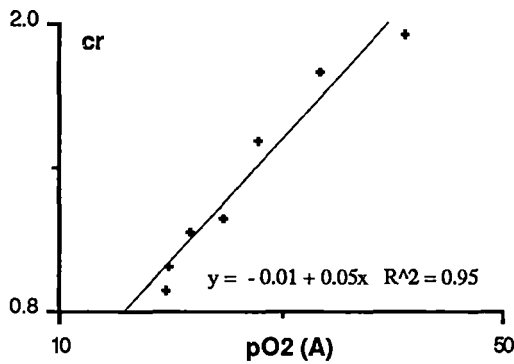
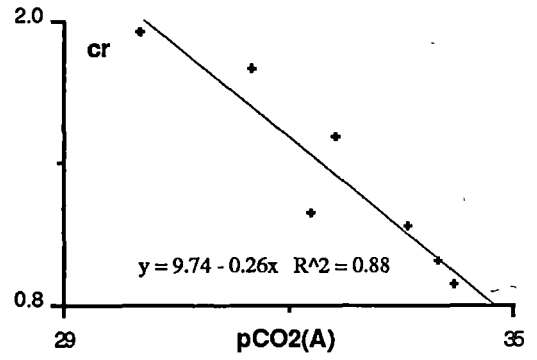
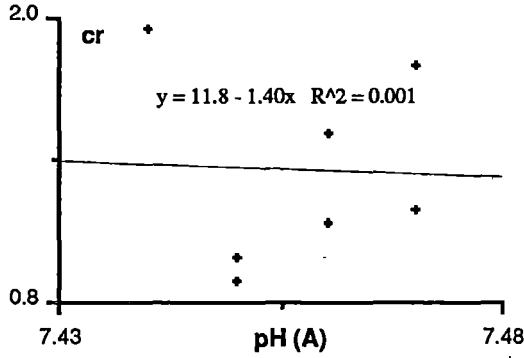


**E:  $\dot{MVO}_2$  (ADO inhibition)**



# 7. Data from experiment 90.11.22

## A: ARTERY (control)



## B: VENOUS (control)

