THE CARDIOVASCULAR CONSEQUENCES OF BURN INJURY

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ABSTRACT

FACULTY OF SCIENCE
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This thesis is concerned with the cardiovascular changes which occur in the first 90 minutes after burn injury. In these studies, a full thickness burn injury of the skin was induced in anaesthetised rats using a method based on those of Arturson (1960) and Philpot (1981).

Burn injury leads to many physiological changes, including falls in the cardiac output, the stroke volume of the heart and the peripheral vascular conductance, but only small changes in blood pressure and heart rate. It has been suggested that the loss of plasma volume due to leakage from damaged or dilated capillaries, leads to a decrease in venous return (i.e preload on the heart) and therefore to a decrease in stroke volume. This thesis, however, presents evidence that the initial event leading to these cardiovascular responses is a decrease in myocardial contractility.

The role of angiotensin II during the early post-burn period was investigated using captopril to block the renin-angiotensin system. Captopril protected against the falls in stroke volume and peripheral conductance. This dependence of stroke volume on peripheral conductance is most easily explained if myocardial function is impaired at the time.

To obtain evidence for this hypothesis I measured cardiac function in the rat directly. I developed a measuring system which uses a fluid-filled blood pressure transducer and a BBC microcomputer to obtain measurements directly from the heart of the rat. Also, I used signal processing techniques to correct for resonance and damping artifacts inherent in such measurements. A new technique called the 'drop test' was devised for measuring the resonance and damping properties of the system. Several pressure-based indices of heart muscle performance were computed from blood pressure measurements recorded from inside the left ventricle of the rat.

The physiological relevance of these indices was then verified by measuring the reproducibility, sensitivity and preload/afterload dependence of the indices. In these studies, the indices derived from the second differential of left ventricular pressure were found to give the most specific indication of changes in contractility.

These indices of myocardial contractility, heart rate, stroke volume and arterial blood pressure were monitored before and after burn injury. The majority of the indices of contractility were depressed by 30-40% at 20 minutes post-burn. This depression was greatest at 30 minutes post-burn, but disappeared by 60 minutes post-burn.

A possible cause of the myocardial depression was investigated in two further studies. Plasma taken from a rat 5 minutes after a burn injury caused a fall in cardiac output when transfused into a recipient rat. This plasma also caused a significant deterioration in the contractile function of an isolated heart muscle preparation.

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ABBREVIATIONS

AII	angiotensin II.
ACE	angiotensin converting enzyme.
APD	energy averaged pressure density.
ARPD	energy averaged rate of pressure density generation.
CO	cardiac output.
CVP	central venous pressure.
dP/dt_{max}	maximum rate of change of pressure.
d^2P/dt^2_{max}	maximum rate of change of dP/dt.
ECF	extra cellular fluid.
ECG	electrocardiograph.
FARPD	frequency normalised, power averaged rate of power density generation.
FFT	fast Fourier transformation.
HR	heart rate.
IP	intraperitoneal.
IV	intravenous.
LVEDP	left ventricular end diastolic pressure.
LVP	left ventricular pressure.
M.A.P.	mean arterial blood pressure.
n	number of observations.
PaO_2	arteriolar partial pressure of oxygen.
$PaCO_2$	arteriolar partial pressure of carbon dioxide.
PC	peripheral conductance.
RAP	right atrial pressure.
SEM	standard error of the mean.
SNS	sympathetic nervous system.
SR	sarcoplasmic reticulum.
SV	stroke volume.
TPR	total peripheral resistance.
V_{CE}	velocity of contractile element shortening.
V_{max}	maximum velocity of muscle fibre shortening.
*	$P < 0.05$.
**	$P < 0.01$.
***	$P < 0.001$.

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INTRODUCTION

INTRODUCTION TO BURN INJURY

This section is a brief introduction to the subject of burn injury.

a) The incidence and mortality of burn injury.

A large burn is probably one of the most severe forms of trauma to which man can be subjected and still survive, albeit with considerable physical and psychological after-effects. In 1975, in England and Wales, the total of deaths due to fire, flame and hot substances was 729 (15 deaths per million of the population). The annual incidence of hospital admissions due to burn injury is between 250 and 420 per million of the population (Muir & Barclay, 1962).

Death or survival after burn injury is dependent on the severity of the burn (depth and area), the age of the patient and the state of health immediately before the injury.

Until the mid 1960's the major cause of death in patients with burns greater than 40% of the body surface area, was infection. However, since the introduction of effective topical anti-infectives, deaths due to infection have decreased significantly, although it still remains an important complication of burn injury (Sevitt, 1979).

Approximately 50% of all burn related deaths still occur within the first 10 days of injury, a significant cause of death being inadequate or inappropriate fluid resuscitation in the treatment of 'burn shock' (Moncrief, 1979). This suggests that investigations into the pathophysiological changes which cause 'burn shock' may become increasingly important in reducing the mortality from burn injury.

b) The types of burn injury.

Skin may be burned by thermal energy applied by radiation, convection or conduction, and the severity of injury depends on the site and duration of exposure, the applied temperature and the efficiency with which the energy is transferred to the skin (Pruitt & Mason, 1979).

Radiation is a fairly inefficient method of burning unless the energy source exceeds 1000°C. The incident energy varies with distance according to the inverse square law, and with the wavelength of the radiation. Common causes of burn injury by radiant energy are electric arc flashes of short duration and high intensity flame burns.

Burning by convection is very dependent on the humidity of the air through which heat is convected, as

dry air has a low heat capacity. Burns caused by a blast of steam are an example of burning by convection.

Conduction, the transfer of energy through a solid or liquid to another surface, is an efficient process. Fortunately the skin is a good thermal insulator, and so heat penetration of the skin is poor. Conduction burns are probably the commonest type of burn injury, including scalds and contact burns.

c) The structure of the skin.

Burn injury is usually incurred at the skin, although underlying areas, and, in some cases, the airways may also be involved. The skin has two main layers, the epidermis and the dermis (Fig.1). The epidermis is the outermost layer and is subdivided into 4 layers or strata: the outermost stratum corneum, the stratum lucidum, the stratum granulosum and the innermost stratum malpighii. The stratum corneum consists of dead, keratinised cells, whilst the inner stratum malpighii contains the germinal epithelium. There is a migration of cells from the continually dividing germinal layer, out to the distal strata, from where the cells are continually lost. In general, the epidermis has no vascular supply but the germinal epithelium is in contact with the dermis and receives its blood supply from this layer.

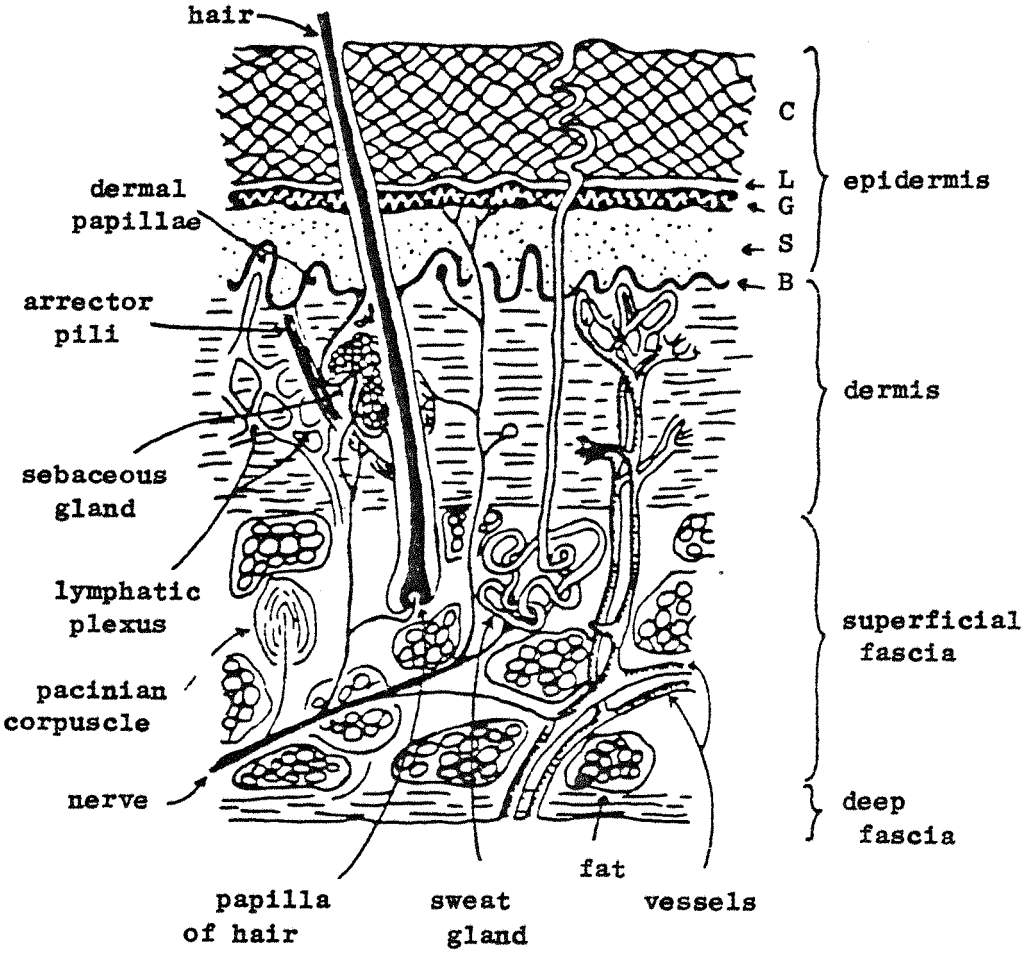
The dermis contains many blood vessels, lymphatic vessels, nerves and nerve endings. The uppermost surface of the dermis is uneven and consists of vascularised papillae which project up into the epidermis. The dermis contains various other structures such as hair follicles and sweat and sebaceous glands. The dermis is secured to the underlying deep fascia by subcutaneous tissue.

The subcutaneous layer contains collagenous and elastic fibres, which project up into the dermis and give the skin structural stability. This layer is also a site for fat storage and clusters of fat globules with a good vascular supplies are often observed. In various places, the skin contains direct connections between arterioles and venules (i.e. arterio-venous shunts), which are important in thermoregulation.

The underlying tissue contains a large network of sensory nerves, mostly with endings in the dermis. These fibres are generally myelinated but some unmyelinated nerves are also found.

d) The classification of burn injuries.

Burn injury can affect the skin with varying severity, ranging from superficial injury to the epidermis, to complete destruction of both epidermis and dermis, with possible injury to underlying fascia, muscle



strata of epidermis:

C = corneum
L = lucidum
G = granulosum
malpighii { S = spinosum
B = basale or germinativum

Fig. 1
The structure of skin.

or organs. It is important to be able to assess the severity of such burn injuries quickly and accurately, as the optimum treatment may depend on the severity of the injury.

The severity of burn injury is classified as partial thickness, full thickness or a mixture of both. This classification is simpler than the old system of first, second and third degree burns, and was introduced to improve the speed and accuracy of estimation of burn wound severity.

Partial thickness burns are characterised by the survival of at least some epidermal elements or dermal papillae containing germinal epithelium. This effectively classifies partial thickness injuries as ones from which new epithelial cells, and therefore, new dermis and epidermis can regenerate. Superficial partial thickness burns may cause blistering of the skin, revealing well vascularised tissue which may be extremely sensitive to touch or temperature. Deeper, partial thickness burns are usually waxy white but still soft and elastic and retain some tactile sensitivity. Partial thickness injuries are commonly caused by electrical injuries, minor scalds or brief exposure to radiant energy or flames.

Full thickness burns are usually dry, hard and inelastic and on exposure to air become dessicated and translucent. This layer of dead skin is called an eschar and can cause compression of underlying tissues, especially in circumferential burns of limbs or the torso when oedema forms beneath it. Because full thickness burns involve complete destruction of the epidermis and dermis, the skin is not capable of regeneration, and all nerve endings are destroyed, therefore inducing anaesthesia in the burn area. These full thickness burns are commonly caused by immersion scalds or by prolonged exposure to flames or intense radiant energy.

e) The response of the skin to heat.

The initial response of skin to heat is an inflammatory reaction, the most obvious sign of which is erythema, a flushing of the skin caused by a localised vasodilatation. This vasodilatation enhances the dissipation of heat from the skin at the site of the reaction. The lower limit for burn injury to the skin, is prolonged exposure to a temperature of 44°C, erythema is induced at about 3-4°C below this temperature. It is interesting that the average temperature of bath water preferred by humans is 40-42°C, close to the limits of injury after long periods of exposure. Fig.2 shows how the induction of erythema varies with temperature and duration

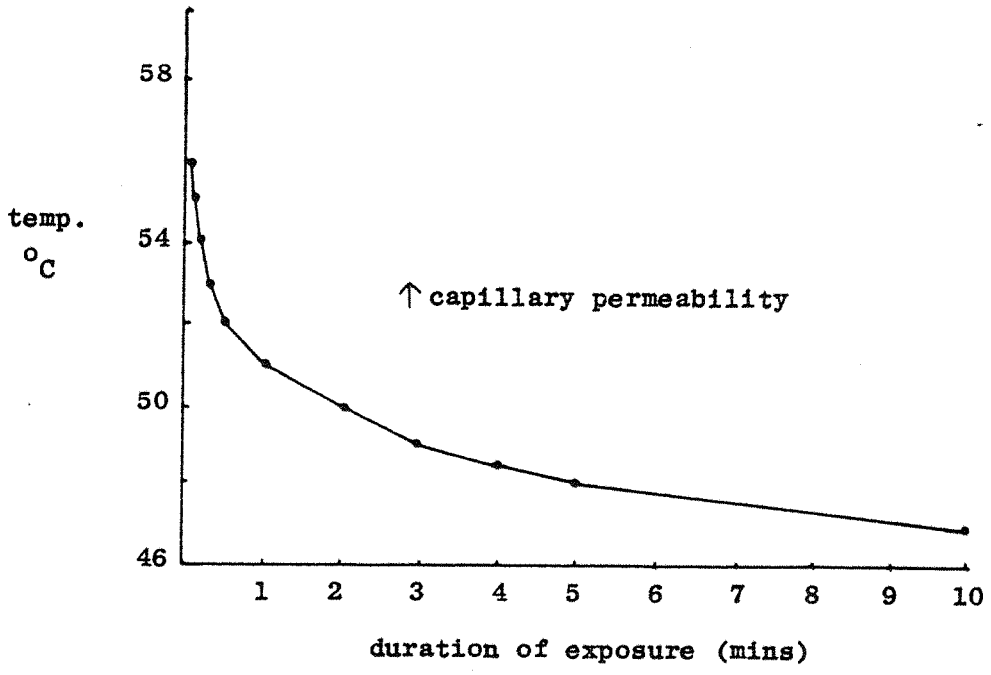


Fig. 2
The threshold for the induction of erythema in relation to the temperature and duration of exposure (redrawn from Davies, 1981).

of exposure. As the exposure to heat intensifies, histamine is released from mast cells and causes an increase in capillary permeability which leads to localised oedema. Between 44 and 51°C the rate of cellular destruction doubles with each 1°C rise in temperature and shorter periods of exposure are necessary to induce such cell damage. Above 70°C full thickness burns can occur in seconds. Further details of the complex pathological and biochemical changes which occur in skin after burn injury are given by Davies (1982).

f) The 'burn shock' syndrome.

Although burn injury usually affects only one organ directly, (i.e. the skin), the consequences of the injury may lead to a form of circulatory shock, the 'burn shock' syndrome (Arturson, 1964). The clinical consequences of shock include: cold, sweaty skin; low systolic blood pressure; hyperventilation and inadequate urine flow leading to uraemia. Initially the patient may be alert, but as the burn shock progresses he becomes disturbed, disorientated and restless. He complains of feeling cold, air hunger and intense thirst. Often consciousness is only lost as a pre-terminal event.

Some of these changes result from the compensatory mechanisms which act to preserve coronary and cerebral perfusion, eventually at the cost of other tissues.

Until recently, burn shock was thought to be caused by hypovolaemia, largely due to the loss of plasma into the interstitium through damaged or leaky capillaries (it is well established that hypovolaemia can cause falls in stroke volume and cardiac output). The fall in cardiac output tends to cause a fall in blood pressure, which is opposed by reflex activation of the sympathetic nervous system i.e. increased heart rate, contractility and vasoconstriction. The progressive fall of cardiac output accompanied by peripheral vasoconstriction inevitably leads to shock, a situation where a vicious cycle is initiated, in which decreasing cardiac output leads to decreased myocardial perfusion, which inevitably leads to further reduction in cardiac output. There comes a point in this circle when even the restitution of blood volume cannot prevent death (Guyton, 1979).

In later chapters of this thesis it will be argued that hypovolaemia is not the sole cause of burn shock.

g) Infection of the burn wound.

After a full thickness burn, the vascular supply to the burned area may become completely occluded, with no appreciable blood flow for up to three weeks. Meanwhile,

the dead tissue provides an excellent anaerobic environment for the growth of bacteria, the primary offending organism being the gram-negative bacterium Pseudomonas Aeruginosa (Teplitz, 1979). The local vascular occlusion prevents the body's humoral and cellular defence mechanisms from dealing with these micro-organisms (Moncrief, 1973). It has also been shown that the post-burn hypermetabolism and increased protease activity, lead to a decrease in immunoglobulin concentration which further reduces the effectiveness of the defence mechanisms.

Infection may also represent a major threat to the patient with partial thickness burns, where the vascular supply is usually disrupted for 24-48 hours. This is long enough for bacteria to invade the vascular supply as it is re-established, causing progressive thrombosis. In this way partial thickness injuries may be converted to full thickness skin loss.

h) The treatment of burn injuries.

A full thickness burn injury to over 15% of the body surface area of adults or 10% of the body surface area of children, requires immediate treatment by fluid resuscitation. The primary treatment is therefore the restitution of blood volume, which should cause an improvement in cardiac output. The seriously burned patient is given a regimen of fluid resuscitation determined by the extent of his injuries. Fluid resuscitation is complicated by the fact that the capillaries become more permeable to molecules with a molecular weight of less than 125,000 (Arturson, 1961), so albumen, a major plasma protein, tends to leak out through the capillaries into the interstitium, thus decreasing the osmotic gradient which normally retains fluid in the circulation. Colloid solutions are usually used for resuscitation in this country. The best fluid is a solution of human plasma protein fraction, but Dextran 70 in normal saline can also be used to boost the osmotic pressure gradient in the capillaries (Brown & Ward, 1984). The volume of fluid to be administered is usually calculated by a formula, of which there are several in use today (see Davies, 1982, chapter 5) and the effectiveness of treatment is monitored by venous haematocrit. Once this has been restored to normal values, urine flow is often regarded as the best indicator of cardiovascular status.

A major problem associated with fluid resuscitation is the possibility of fluid overload. As the capillary hydrostatic pressure increases, the formation of oedema is

enhanced and the patient is in danger of developing pulmonary oedema.

The problem of infection must also be dealt with rapidly, and topical anti-infectives are usually applied. Common topical anti-infectives include silver nitrate and silver sulphadiazine. The major problem with topical anti-infectives is their inability to penetrate deep wounds, but since the local vascular supply is often lost, there is little alternative.

A new approach to the fight against infection, is the development of a vaccine against pseudomonas bacteria. PEV 01 is a polyvalent pseudomonas vaccine which is effective against many strains of psuedomonas aeruginosa and has been found to be a useful aid to antibiotic therapy (Jones et al, 1976).

The longer term treatment for full thickness burn injuries is plastic surgery, usually the grafting of skin from one site on the body onto the excised burn wound (i.e a homograft). If the area of the wound is so large that the amount of donor graft sites is insufficient, other alternatives include temporary coverage of the wound with porcine skin (i.e a heterograft) or artificial skin substitutes. These are not permanent but allow time for new donor sites to become available. An exciting new development is that of autologous cultured human epithelial cells. Successful grafts have been produced after cells from a small skin biopsy have been cultured in vitro and then applied to the wound (Gallico et al, 1984). The obvious advantage of this over other methods, is that these grafts are autologous and are therefore seldom rejected.

CHAPTER 1:
General cardiovascular function after burn injury.

1.1 INTRODUCTION

After severe burn injury, the cardiac output is thought to fall within a few minutes, long before burned patients can be examined. It is therefore necessary to study these changes in experimental animals. In an experimental animal preparation, measurements of cardiac output can be obtained at frequent intervals, before and after a standard burn injury, allowing each animal to act as its own control.

In the immediate post-burn period there is an increase in the total peripheral resistance of the vasculature (Michie *et al*, 1963) and Banner *et al*, (1980) have shown that this contributes to the fall in cardiac output. Philpot (1981) then suggested that the renin-angiotensin system may be involved in this change in TPR, since her studies showed a 400% elevation in plasma renin activity in the rat after burn injury and that nephrectomy greatly diminished the fall in cardiac output caused by burn injury.

This chapter is concerned with the effects of burn injury on cardiovascular function, especially cardiac output and peripheral resistance. The factors which normally influence cardiac output and the effects of burn injury are briefly reviewed. It is suggested that the renin - angiotensin II system may be involved in altering the cardiac output and peripheral resistance after burn injury. The importance of the renin-angiotensin system in the cardiovascular responses to burn injury was investigated experimentally by studying the effects of various blockers of the renin-angiotensin system.

1.2 LITERATURE REVIEW.

1.2.1 The control of cardiac output.

The cardiac output is defined as the volume of blood pumped by the left ventricle per unit time.

The cardiac output is normally dependent on the following 4 factors, although there may be considerable interaction between them:-

a) Heart rate.

The frequency of contraction of the myocardium is determined by the pacemaker cells of the sino-atrial node, which is situated in the wall of the right atrium. These cells possess an intrinsic rhythmicity which can be altered by the neurotransmitters noradrenaline and acetylcholine, by variations in the levels of plasma ions and by the degree of atrial stretch.

Sympathetic nervous system activity causes the release of adrenaline from the adrenal medulla and noradrenaline from nerve terminals in the sino-atrial node. These neurotransmitters act directly on β-adrenoreceptors causing activation of the c-AMP mediated secondary messenger system, leading to an increase in the rate of diastolic depolarisation and therefore the rate of production of action potentials. This is brought about by potentiation of the slow inward current carried by sodium ions and inactivation of the fast outward current carried by potassium ions (Carmeliet & Vereecke, 1979). Vagal stimulation exerts a negative chronotropic effect due to acetylcholine release from parasympathetic nerves onto the S.A node. Muscarinic receptor activation leads to a hyperpolarisation of the cell membrane as well as a decrease in the rate of diastolic depolarisation (Levy, Martin & Stuesse, 1981). There are also changes in the rate of conduction in the A.V. node during autonomic nervous system stimulation (Levy et al, 1981).

The sensory input which causes reflex changes in heart rate on a beat to beat basis, originates from the arterial baroreceptors (Smythe et al, 1969), and from the atria (Linden & Kappagoda, 1982).

In normal animals or individuals, changes in cardiac output are usually accompanied by changes in heart rate so that stroke volume remains fairly constant, but if the heart is artificially paced then reflex alterations in contractility and preload can leave cardiac output unaltered (Braunwald & Ross, 1979). At high heart rates however, the cardiac output falls because the brevity of diastole reduces ventricular filling and also reduces

myocardial perfusion, which occurs during diastole (Braunwald & Ross, 1979).

b) Preload on the heart.

The preload on the heart is defined as the tension in the wall of the left ventricle at the end of diastole (Braunwald & Ross, 1979). In most studies, however, the left ventricular end diastolic pressure (LVEDP), right atrial pressure (RAP) or central venous pressure (CVP) is taken as an index of the preload on the heart.

The stroke volume and the LVEDP are related by the Frank-Starling law of the heart (Starling, 1918). This states that "the mechanical energy set free on passage from the resting to the active state is a function of the length of the muscle fibre", (Fig.1.1). Over the physiological range, when other factors are held constant the quantity of blood ejected from the heart is proportional to LVEDP. This property of the heart is due to an intrinsic intraventricular mechanism which is not neural or humoral in origin and cannot solely be explained by changes in the overlap of actin and myosin filaments, but may involve changes in intracellular calcium availability (Noble, 1978).

There are many factors which can alter the preload on the heart. The venous return is determined by the interaction between the forces returning blood from the peripheral circulation to the heart and the heart's ability to pump the blood it receives (Guyton 1981). The peripheral circulation and its capacitance therefore greatly influence the preload on the heart. A rapid reduction of blood volume will cause a decrease in cardiac output but a slow reduction of blood volume may be compensated for by mechanisms activated by the sympathetic nervous system. The distribution of blood within the circulatory system is an important factor and can be modified by many factors, including:-

i) Venous tone. The smooth muscle in the walls of all venules responds to a variety of neural and humoral stimuli (Braunwald & Ross, 1979). Venoconstriction acts to increase the intrathoracic blood volume and augment the venous return. It is probable that this mechanism plays a major part in the regulation of cardiac output.

ii) Posture. Standing decreases the intra-thoracic blood volume and therefore ventricular end diastolic volume resulting in a decreased venous return and cardiac output.

iii) Respiration. Intrathoracic pressure varies in a cyclical manner during respiration, decreasing

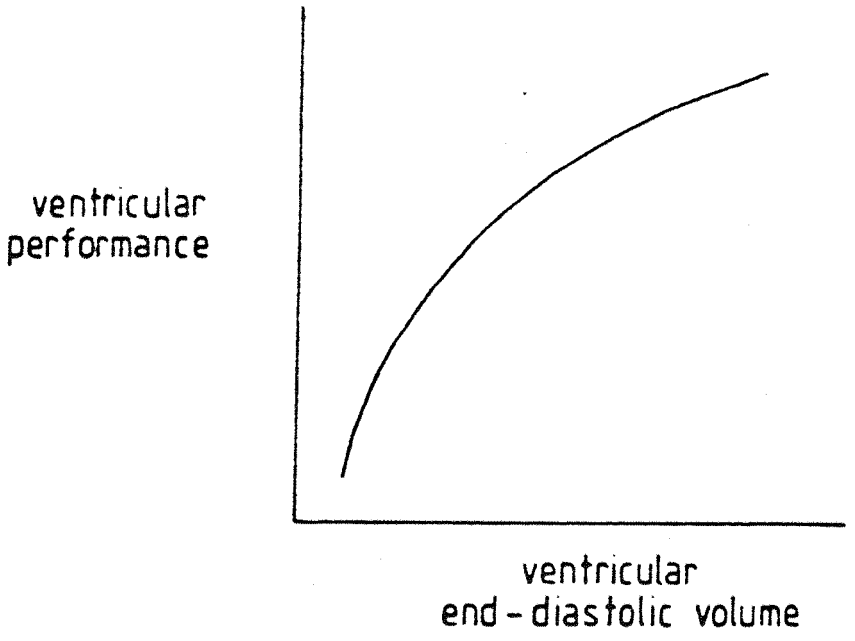


Fig. 1.1
The relationship between ventricular performance and end-diastolic volume (the Frank-Starling curve).

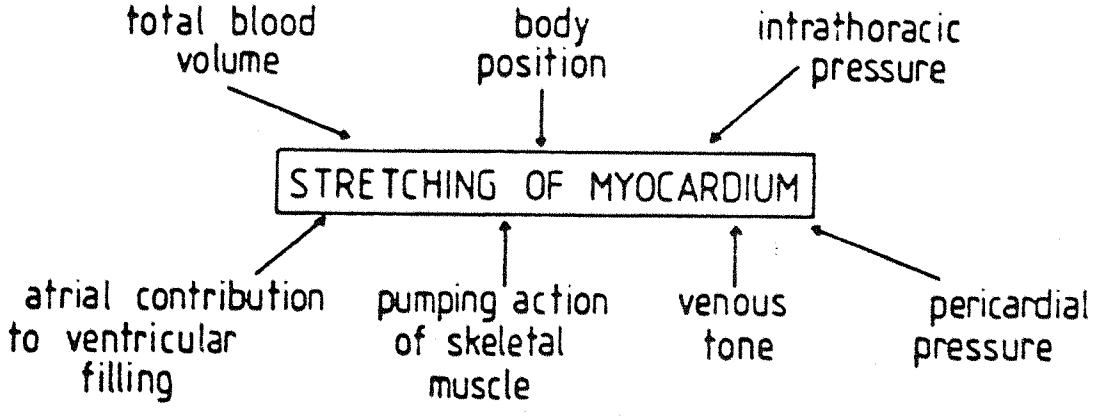


Fig. 1.2
The major factors which affect end-diastolic volume of the heart (from Braunwald, Ross and Sonnenblick, 1967).

during inspiration and increasing during expiration. The greater the intrathoracic pressure, the more venous return is impeded.

iv) The atrial contribution to diastolic filling.

v) Blood volume.

vi) The muscle pump.

vii) Intra-pericardial pressure

These factors are summarised in Fig.1.2. The the most important of these in the control of the preload on the heart is the venous capacitance. For example, a fall in arterial blood pressure activates the arterial baroreceptors which cause sympathetic nervous stimulation of venous tone.

c) Afterload on the heart.

The concept of the afterload on the heart is one which is difficult to define in a way that clearly carries the intended meaning i.e. the force or pressure which opposes the ejection of blood from the contracting ventricle. Braunwald & Ross (1979) define the afterload as "the tension in the wall of the ventricle at the end of systole". The blood pressure in the ventricle at the end of systole can be taken as an index of ventricular wall tension and hence the afterload. The mean aortic blood pressure is also used as a measure of the afterload. The aortic impedance (i.e the impedance of the circulatory system starting from the aorta), is a factor which clearly influences the afterload on the heart and this impedance has two components: the distensibility of the arterial walls and the total peripheral resistance to blood flow (TPR).

An increase in arterial blood pressure does not, however, necessarily reduce the stroke volume of a normal heart but elicits an increase in the contractile state of the myocardium known as homeometric autoregulation or the Anrep effect (Anrep, 1912), which tends to maintain stroke volume and cardiac output (Sarnoff & Mitchell, 1962). In states of ventricular dysfunction, such as heart failure, the afterload becomes a more important determinant of cardiac output (Cohn & Franciosa, 1977).

Arterial blood pressure, and hence the afterload on the heart, is influenced by changes in total peripheral resistance, which in turn is regulated by the sympathetic nervous system and also by other factors such as angiotensin II, adrenaline and the local control mechanisms which normally ensure that tissue perfusion matches metabolic requirements.

d) Contractility.

The contractility of the heart is defined as "the intrinsic ability of the heart to function other than that induced by changes in pre-systolic fibre length" (Rothe, 1976). There are many factors which affect the contractility of the heart, some intrinsic and some extrinsic. These factors are discussed in chapter 3.

In heart failure, the ventricular contractility is often the factor limiting cardiac performance (Braunwald & Ross, 1979).

1.2.2 Cardiovascular changes after burn injury.

It is now well established that there is a fall in cardiac output after extensive burn injury. This has been observed in studies on burned patients (Pruitt et al, 1971) and also in numerous studies on experimental animals (Dobson & Warner, 1957; Gilmore & Handford, 1956; Fozzard, 1961; Michie et al, 1963; Okamoto et al, 1974; Turner et al 1977; Philpot, 1981).

For example, Moncrief (1966) observed that after a scald injury covering 50% of the body surface area of the anaesthetised dog, the cardiac output was decreased by 50% within a few minutes, 60-70% after one hour and continued to fall until death occurred. The lowered cardiac output is probably an initiating factor of the 'burn shock syndrome ' (Arturson, 1961).

The decrease in cardiac output leads to a reduction in the perfusion of the tissues. Ferguson et al (1977) showed a decrease in absolute blood flow to all organs except the adrenals and liver in the burn injured guinea pig. The brain and the myocardium received increased proportions of the cardiac output but nevertheless a fall in actual blood flow.

The initial cause of this fall in cardiac output is a controversial subject and the possible factors involved are discussed below.

a) Preload.

It is well established that there is a fall in plasma volume after burn injury due to oedema formation and this fall in plasma volume causes an increase in haematocrit (Underhill et al, 1923; Blalock, 1930). The use of radio-labelled albumen and erythrocytes has allowed more accurate measurements of blood and plasma volumes and such studies have shown that the loss of plasma is proportional to the area and depth of the burn and that up to 50% of the plasma volume may be lost from the circulation (Davies, 1982).

The post-burn formation of oedema is due to an

increase in capillary permeability and local vasodilatation. These changes have been suggested to be caused by several mechanisms:

i) Heat induced capillary damage at the site of injury (Cuppage et al, 1973).

ii) The release of vaso-active substances. It has been established that histamine, serotonin, bradykinin and prostaglandins (E and F2α) are all present in elevated concentrations in burned skin and blister fluid soon after burn injury (Wells & Mills, 1963).

iii) Neural influence. The 'axon-reflex' has been shown to play a part in post-burn oedema, since capsaicin pre-treatment, which causes the degeneration of primary afferent C-fibres, diminished the plasma volume loss after burn injury (Saria & Lundberg, 1983).

iv) Superoxide radical formation. An interesting new hypothesis implicates the production of free radicals in the formation of oedema. Several different sources have been suggested for the production of these free radicals: the degradation of purine nucleotides (Saez et al, 1983), the metabolism of polyunsaturated free fatty acids via the cyclo-oxygenase pathways (Hilton, 1980) and leucocytes (Root & Metcalf,1977).

This fall in blood volume may be a major factor in the development of burn shock. Davies (1982) asserts that when blood volume measurements from various studies are extrapolated back to the time of burn injury, the consensus of the results shows the falls in cardiac output and blood volume to be virtually simultaneous. However, much evidence shows that cardiac output decreases before any significant change in central venous pressure, suggesting that hypovolaemia is not the only factor responsible for the fall in cardiac output (Baxter et al, 1966; Gilmore & Handford, 1956; Turner et al, 1977 ; Kuzin et al 1983).

After a burn injury, fluid resuscitation can return cardiac output to normal levels, but this does not necessarily mean that the low cardiac output is caused by hypovolaemia, since infusion of fluid into non-burned subjects also causes an increase in cardiac output. Furthermore, the cardiac output may return to normal levels before blood volume is restored in burn injured patients (Moncrief, 1966). In burn injured animals an arterio-venous shunt, which restored the preload on the heart, did not prevent the reduction of cardiac output

(Moncrief, 1966). Also pharmacological agents can prevent the formation of oedema but cannot prevent the fall in cardiac output (Hilton, 1980; Philpot, 1983).

All of this evidence suggests that the fall in cardiac output is initially independent of the preload on the heart. The preload on the heart is obviously an important factor contributing to the fall in cardiac output in the long term, but probably not in the immediate post-burn period.

b) Afterload.

The afterload on the heart has two determinants, the aortic compliance and the impedance or total peripheral resistance of the vasculature (TPR). Burn injury causes an increase in TPR which is not associated with any significant change in arterial blood pressure (Michie et al, 1963; Moncrief, 1966; Turner et al, 1977; Len'kova, 1974 ; Philpot, 1981). There is evidence implicating the elevation of TPR with the depression of cardiac output during the early post-burn period. Sympathetic blockade with chlorisondamine after burn injury caused simultaneous improvement in cardiac output and reduction in TPR (Turner et al, 1977). Pre-treatment of rats with the ganglion blocker pentolinium prevented the changes in TPR and cardiac output (Banner, 1980), and the vasodilator hydralazine reduced the changes in TPR and cardiac output in burn injured rats (Pagdin, 1980), and also in dogs (Moncrief, 1966).

It is difficult to ascertain whether the increase in TPR is the cause of the depression of cardiac output or a reflex response to protect the blood pressure from falling when cardiac output decreases. Michie et al, (1963) suggested that burn injury causes peripheral vasoconstriction and vascular obstruction which increase the TPR, thus increasing the afterload on the heart and opposing cardiac emptying. An objection to this hypothesis, however, is that by definition, an increase in afterload on the heart must be accompanied by an increase in aortic blood pressure and as previously mentioned this does not generally occur after burn injury. A different example may make this point clearer: in a normal animal, the potent vasoconstrictor substance angiotensin II causes an increase in TPR and hence arterial blood pressure (De Bono, 1963); as a consequence of the increased blood pressure there is a fall in cardiac output perhaps due to this increased afterload on the heart but also due to reflexes arising from the arterial baroreceptors. Furthermore in normal hearts the cardiac output is not necessarily sensitive to small changes in afterload

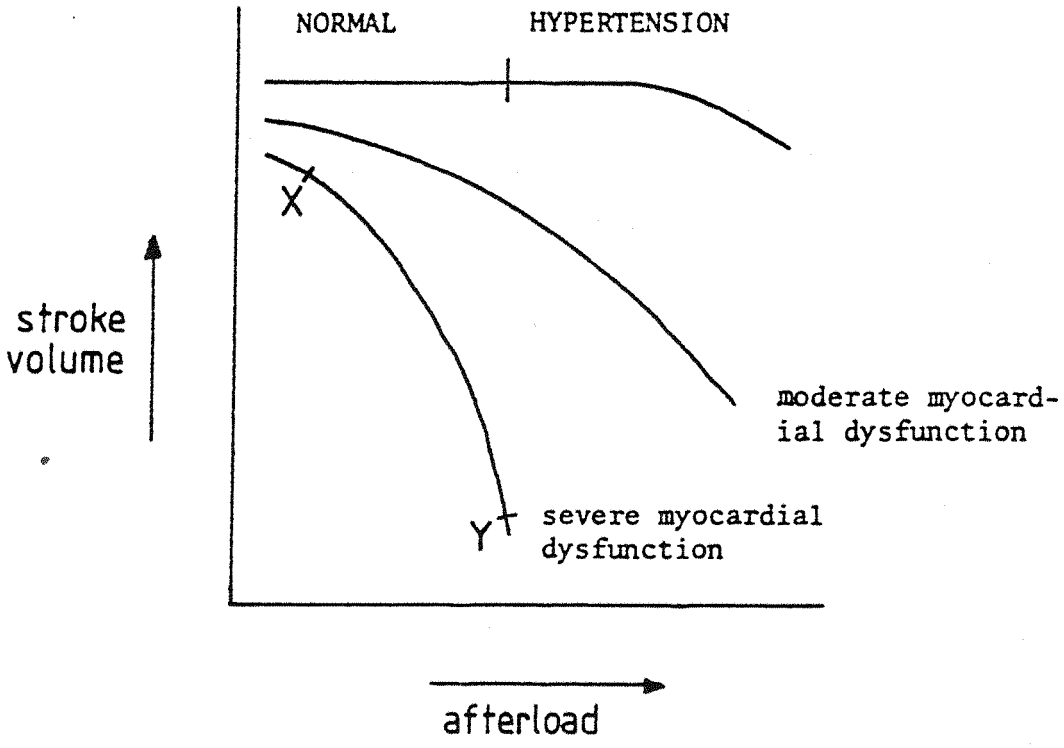
because of homeometric autoregulation. Thus Michie's proposal is, by itself, unable to explain the fall in cardiac output: since there is no rise in aortic pressure there is no rise in afterload perceived by the heart.

An alternative possibility is that the increase in TPR is a reflex response to halt the decline in blood pressure caused by a sudden depression of ventricular function after burn injury (Len'Kova, 1979; Philpot, 1981). Any such impairment of the ventricle would alter the functional characteristics of the heart, and it has been shown that the failing heart is very sensitive to changes in afterload (Cohn & Franciosa, 1977), (see Fig.1.3). A failing or diseased heart will tend to show a decreased stroke volume (and hence cardiac output) when presented with an increased afterload.

The artificial lowering of TPR in the studies named above generally causes a decrease in arterial blood pressure i.e. a decrease in the pressure against which the heart is trying to expel blood. It may, therefore, be this fall in afterload which permitted the output of the (putatively) damaged heart to increase in these studies. Shik & Len'kova (1979) reported that anaesthetised rabbits with denervated arterial baroreceptors showed no increase in TPR after burn injury, but observed that the arterial blood pressure fell rapidly. They therefore concluded that the increase in TPR was a reflex mechanism to protect against a fall in blood pressure.

Peripheral vasoconstriction could be brought about by direct stimulation of the arterioles via sympathetic nerves or by circulating vaso-active substances such as adrenaline, angiotensin II, vasopressin and thromboxane. Adrenalectomised, burn injured rats showed a smaller depression of cardiac output but still showed a large increase in TPR (Philpot, 1981). The involvement of angiotensin II in the increase in TPR has been suggested by Philpot (1981), who observed that nephrectomy prevented the increase in TPR after burn injury in anaesthetised rats and that plasma renin activity increased soon after burn injury. This, however does not prove that the renin-angiotensin system is necessarily responsible, wholly or in part, for the rise in the TPR. It would be necessary to establish more directly that the angiotensin II levels were indeed causing vasoconstriction, and this problem was studied in the work reported in this thesis.

Another factor which tends to increase the afterload on the heart is the increased viscosity of blood after a burn injury. Schoen *et al*, (1971) have shown



If cardiac function is normal, then an increase in afterload causes an increase in blood pressure but the stroke volume remains constant.

If cardiac function is impaired, then the blood pressure is no longer determined by afterload since the stroke volume and afterload are inversely related. Consequently, an increase in afterload causes a decrease in stroke volume but the blood pressure may remain unchanged (X→Y).

Fig. 1.3
The relationship between left ventricular stroke volume and peripheral resistance in normal and diseased hearts (from Cohn & Franciosa, 1977)

that there is a significant increase in the viscosity of blood: by 50% at 90 minutes post-burn. During this time the venous haematocrit increased from 39% to 46%. The increase in viscosity was caused mainly by loss of circulating plasma volume, the consequent rise in haematocrit and to a lesser extent by hyperproteinaemia. The increased viscosity of blood may therefore contribute significantly to the increase in TPR and hence lowered cardiac output (Davies, 1982).

c) Heart rate.

The heart rate remains relatively stable after burn injury (Gilmore, 1957; Wolfe & Miller, 1976; Michie, 1963), and the fall in cardiac output is associated with a decrease in stroke volume. Thus the heart rate does not appear to be an important factor influencing cardiac output after burn injury.

The ability of the heart rate to vary after burn injury does not diminish, as post-burn infusion of dopamine caused a substantial increase in heart rate (Philpot, 1981), as does isoprenaline (Aggarwal, 1983) and both atropine and vagal stimulation caused similar decreases in the heart rates of burned and mock-burned rats (Aggarwal, 1983). It is interesting to note that after most other forms of circulatory shock an increase in heart rate is observed (Guyton, 1981).

d) Myocardial contractility.

Myocardial contractility has long been implicated in the burn shock syndrome although there is still great debate concerning the possibility of an immediate post-burn depression of myocardial contractility and its involvement in the depression of cardiac output. This topic is discussed in detail in chapter 4.

1.2.3 The measurement of stroke volume by impedance cardiography.

Impedance cardiography is a non-invasive method for measuring stroke volume developed by Kubicek *et al*, (1966). It does not have a strong theoretical basis and should therefore be considered more as an empirical method of measurement. This method has been validated empirically against other methods for the determination of stroke volume and hence cardiac output. These studies include comparisons with thermo-dilution and electro-magnetic flow meter measurements in anaesthetised rats (Chapman *et al*, 1977) and clinical studies in man (Lababidi *et al*, 1970). The accuracy of the impedance method after burn injury has also been validated in the rat (Griffiths *et al*, 1981). The methodology of the impedance system

used in this laboratory has been validated before and after burn injury by Philpot, (1981). Impedance cardiography has many advantages when used for the measurement of cardiovascular function after burn injury: it is non invasive and does not require the injection or withdrawal of fluids, measurements are quick and easy to obtain and can be repeated at frequent intervals.

1.2.4 The renin-angiotensin system.

The renin-angiotensin cascade system is shown in Fig.1.4. Renin is a specific carboxy-protease whose only known physiological action is to cleave the leucine-leucine bond in its substrate, angiotensinogen to form angiotensin I. The decapeptide angiotensin I is then converted to an octapeptide, angiotensin II, by an enzyme called angiotensin converting enzyme (ACE), found mainly in the lungs. Angiotensin II is further degraded to angiotensin III, a septapeptide which is also known to possess biological activity. Two aspects of the renin angiotensin system are discussed here: the control of renin release and the physiological actions of angiotensin II.

a) The control of renin release.

The factors involved in the release of renin from the renal juxta-glomerular apparatus have been reviewed by Keeton & Campbell, (1980), and are summarised below:-

i) A renal vascular receptor that responds to changes in wall tension in the afferent arterioles.

ii) A macula densa receptor which detects changes in the sodium concentration in the distal tubular fluid.

iii) A negative feedback mechanism of circulating angiotensin II on renin release.

iv) Sympathetic nervous system activation via B-adrenoreceptors on the juxta-glomerular apparatus. The renal nerves are involved in several reflex loops involving cardio-pulmonary receptors situated in the atria and ventricles. When activated by stretch, these receptors lead to an inhibition of renal nerve activity and therefore of renin release. These reflexes are capable of responding to small decreases in blood volume or pressure.

b) The actions of angiotensin II.

i) Angiotensin II is the principle effector hormone of the renin-angiotensin system. It is the most potent pressor substance known, and has been shown to be 10 times more potent on a weight for weight basis,

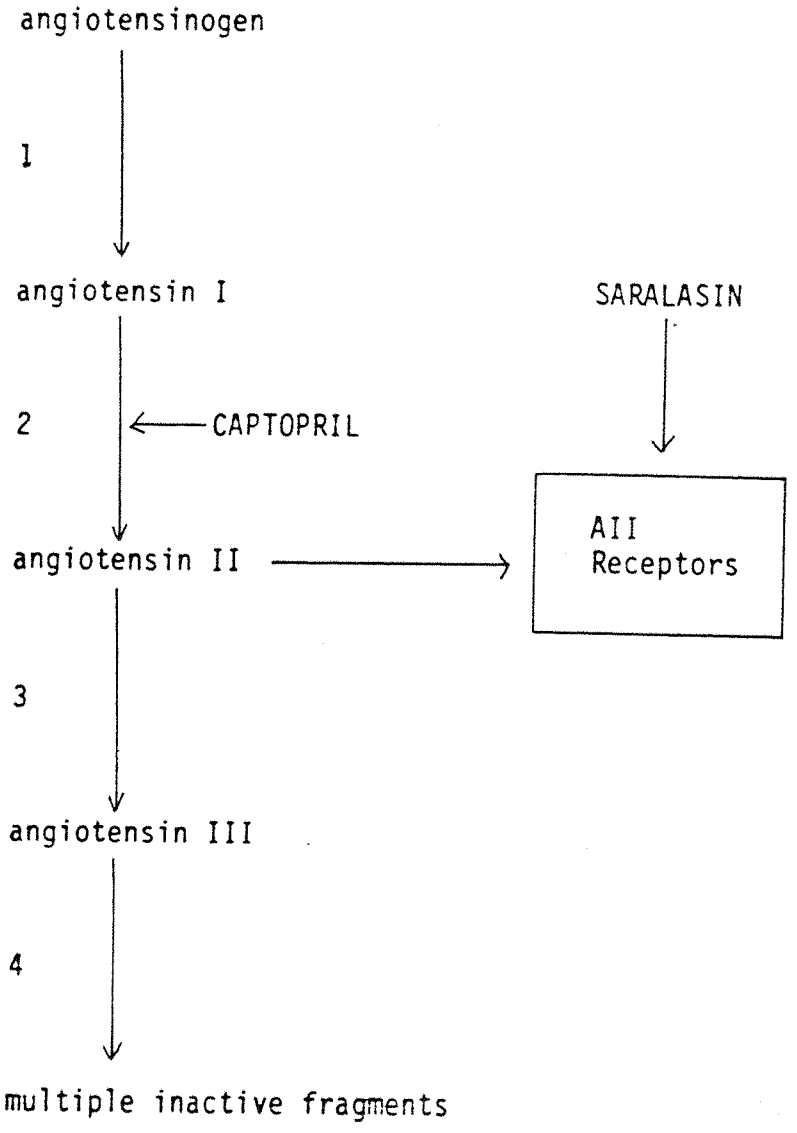


Figure 1.4 The Renin-angiotensin Cascade System and the Site of Action of Inhibitors.

The enzymes involved are:

1 - Renin
2 - Angiotensin converting enzyne (A.C.E.)
3 - Amino-peptidase
4 - Assorted peptidases

than noradrenaline (Gross & Turrian, 1960). It causes a rise in blood pressure by increasing the total peripheral resistance (i.e. by causing arteriolar constriction). This pressor action is due mostly to a direct action on the arterioles (Palaic & Lemorvan, 1971), but another component of the pressor action of angiotensin II is centrally mediated (Bickerton & Buckley, 1961). The central site of the pressor action is believed to be the area postrema of the medulla, an area where the blood brain barrier is relatively permeable. Much evidence also suggests that the angiotensin II induced release of catecholamines from the adrenal medulla contributes to the pressor action of angiotensin II. Cline (1981) has reported that there is a dose dependent contribution of the released catecholamines to the pressor response to angiotensin II in the anaesthetised dog.

ii) The renin-angiotensin system is one of the primary mechanisms in the control of aldosterone secretion and hence the body fluid volume. (Ganong, Mulrow, Boryczka and Cera, 1962). Angiotensin II is believed to stimulate the first step in the biosynthesis of aldosterone, i.e. the conversion of cholesterol to pregnenolone (Aguilera and Marusic, 1971).

iii) Angiotensin II is known to stimulate the release of ADH and ACTH (Ramsey, Keil, Sharpe and Shinsako, 1978)

iv) Another central action of angiotensin II which is important in the control of ECF volume is its dipsogenic action. It has been shown that administration of angiotensin II into the central nervous system stimulates drinking behavior in several species (Fitzsimons, 1972).

v) Angiotensin II can cause both positive inotropic and chronotropic effects on the heart. There is some evidence that these effects of angiotensin II may be mediated by the action of the hormone on the cardiac sympathetic innervation and ganglia.

vi) Angiotensin II causes the stimulation of salt and water transport across epithelial tissues, especially those of the intestine. Sodium and water conservation by the intestine may assist the established salt and water retaining actions of this hormone on the kidney, to make the renin-angiotensin system of great importance in regulating the body's sodium.

c) Inhibitors of the renin-angiotensin system.

There are several inhibitors of the renin-angiotensin cascade system which are commonly used.

i) Captopril, formerly known as SQ14225, (3-mercapto-2-methyl-propanoyl-1-proline), is a competitive inhibitor of the proteolytic enzyme known either as angiotensin converting enzyme (ACE), or kininase II (Rubin et al,1977). Captopril inhibits the conversion of angiotensin I to angiotensin II (see Fig.1.4). This drug is used clinically as an anti-hypertensive agent.

ii) Saralasin (sarcosine-1, alanine-8, angiotensin) is an antagonist for the angiotensin receptor (Pals et al, 1971). This compound is not used therapeutically, but is often used to assay for endogenous angiotensin action.

1.3 MATERIALS & METHODS

1.3.1 The preparation of animals.

Female Wistar albino rats (240-260g) were anaesthetised with sodium pentobarbitone (Sagatal 60mg/kg I.P. plus supplementary doses of 3mg/kg as required) and tracheostomised with polyethylene tubing (Portex 800/100/420). Further preparative procedures for particular experiments are described below, as are the procedures for causing burn injury and mock-burn injury. Animals were sacrificed at the end of each experiment.

1.3.2 The burn injury.

a) Application of a standard burn injury.

The following method of applying a burn injury to an animal is based on the method described by Arturson (1964) and modified by Philpot (1982). The area to be subjected to the burn injury, (in most cases the back), was shaved and dipped into water at 90°C for exactly 20 seconds. The burned area was cooled in water at room temperature for 60 seconds to stop further injury, then dried.

The burn injury used in these experiments produced a burn injury which covered 25-30% body surface area. The injury was applied using a custom made 'burn cradle' (Fig.1.5), which exposed a constant area of the back of the rat to the hot water. The tail was not exposed to heat during this procedure.

b) Measurement of the burn size.

The size of the small burn injury was always measured. The outline of the injury was traced onto transparent polythene and the area measured with a planimeter. The total body surface area was calculated using equation (1) due to Lee, (1929):

(1) $$\text{Surface Area} = k \cdot Wt^{0.6}$$

where W = Body weight in grams
k = 12.44 for a female rat

The area of the large burn was estimated to be 40-60% of the total body surface area.

c) Mock-burn injury.

It is important to have appropriate control animals for comparison with burn injured rats. In this thesis the control procedure is called a mock-burn injury.

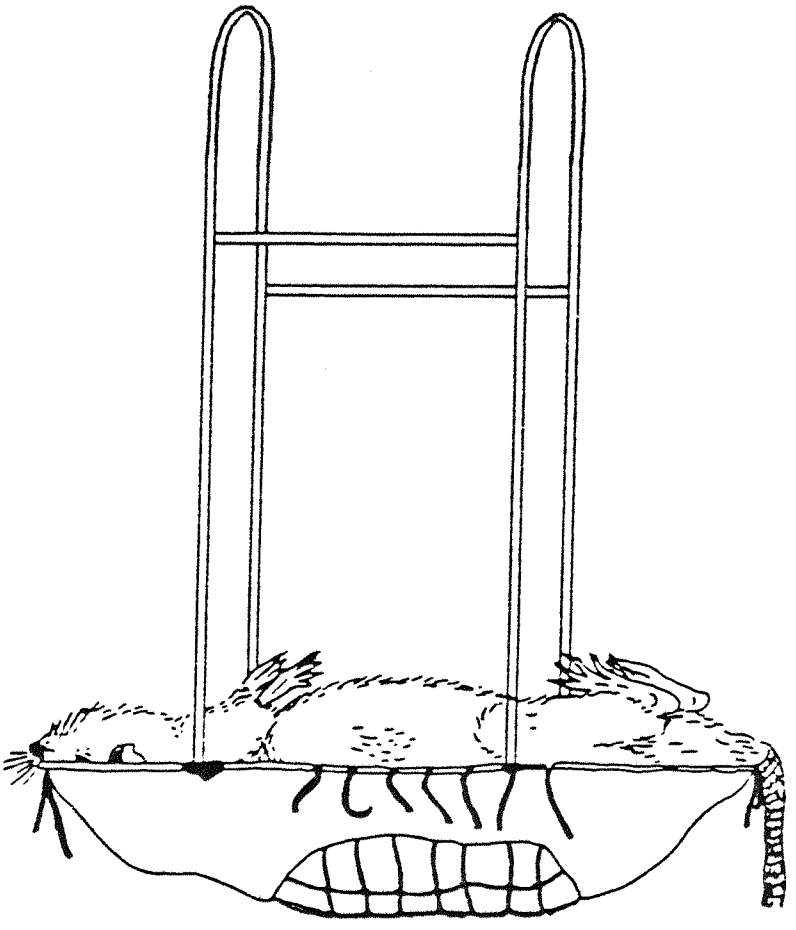


Fig. 1.5
The plastic cradle designed to produce a 20-25% full thickness burn injury to the shaved back of the anaesthetised rat. The actual area of the burn could be varied by adjustment of the tension in the threads.

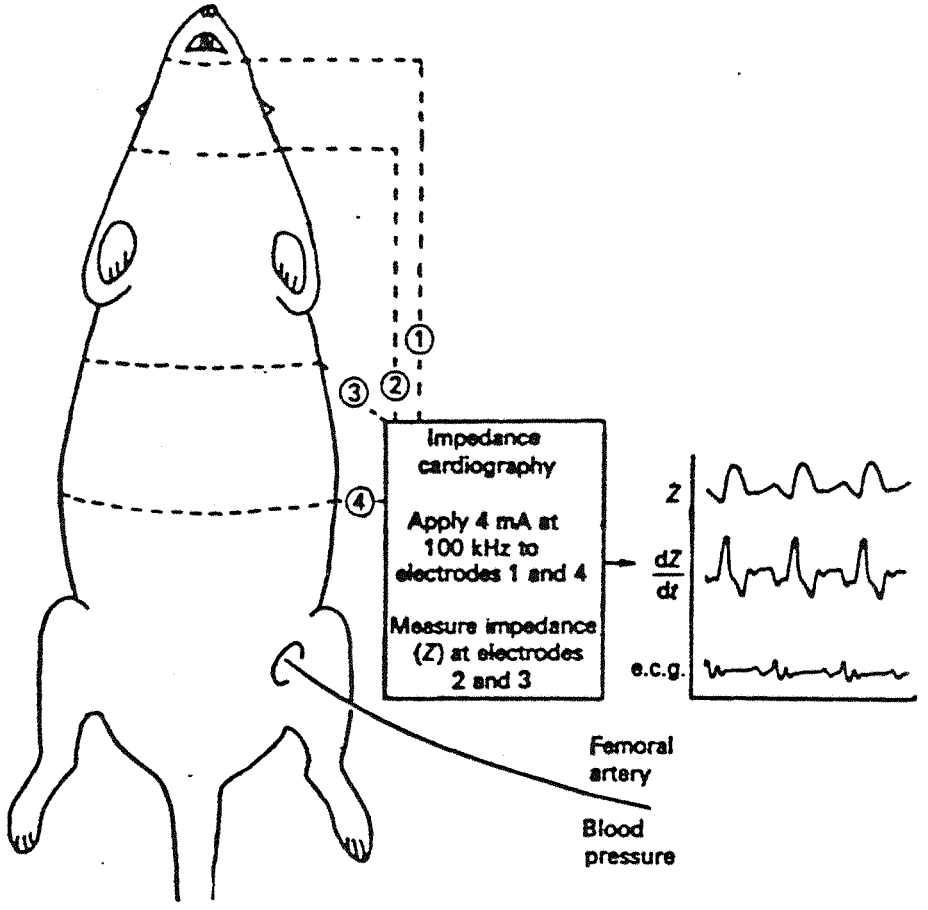


Fig. 1.6
The use of impedance cardiography in the rat, showing the positioning of the four circumferential wire electrodes.

Rats receiving a mock-burn were treated in exactly the same way as rats receiving a burn injury, except that they were lowered into water at room temperature instead of at 90°C. When observing changes due to burn injury, comparisons were made between burn injured rats and mock-burned rats.

1.3.3 The measurement of cardiovascular function.

Stroke volume was measured by impedance cardiography. Four stainless steel wires were placed subcutaneously around the rat using a suture needle. The two ends of each wire were joined to make four circumferential electrodes (Fig.1.6). The electrodes were connected to an Impedance Cardiograph (Minnesota 304A) which passed a small alternating current (4mA at 100kHz) between the two outer electrodes. The cardiograph derived the trans-thoracic impedance (Z) and the ECG from the voltage signals detected between the inner two electrodes. The cardiograph also differentiated the Z signal to produce dZ/dt. These three signals were recorded on a high speed chart recorder (Mingograf 34) at a speed of 100mm/sec.

Stroke volume was calculated using equation (2), due to Lababidi et al, (1969):

(2) $$\text{Stroke volume} = \rho \cdot \left(L/Z \right)^2 \cdot \text{LVET} \cdot (dZ/dt)_{max}$$

where:

ρ = The resistivity of blood at 100kHz (ohms/cm)

L = The distance between the recording electrodes (cm).

Z = The average basal trans-thoracic impedance (ohm).

LVET = The left ventricular ejection time (sec).

dZ/dt_{max} = The maximum rate of change of impedance (ohms/sec).

The relationship between ρ and the haematocrit value is shown in Fig.1.7. The haematocrit was determined whenever stroke volume was measured, by collecting small volumes of blood (0.05ml) in duplicate from the femoral artery into heparinised capillary tubes. Samples were immediately spun down in a haematocrit centrifuge and read in a haematocrit reader.

Measurements of stroke volume were calculated as the average of three consecutive heartbeats.

The heart rate was calculated from the R-R interval of the ECG signal. Three consecutive heartbeats were used to calculate each heart rate measurement.

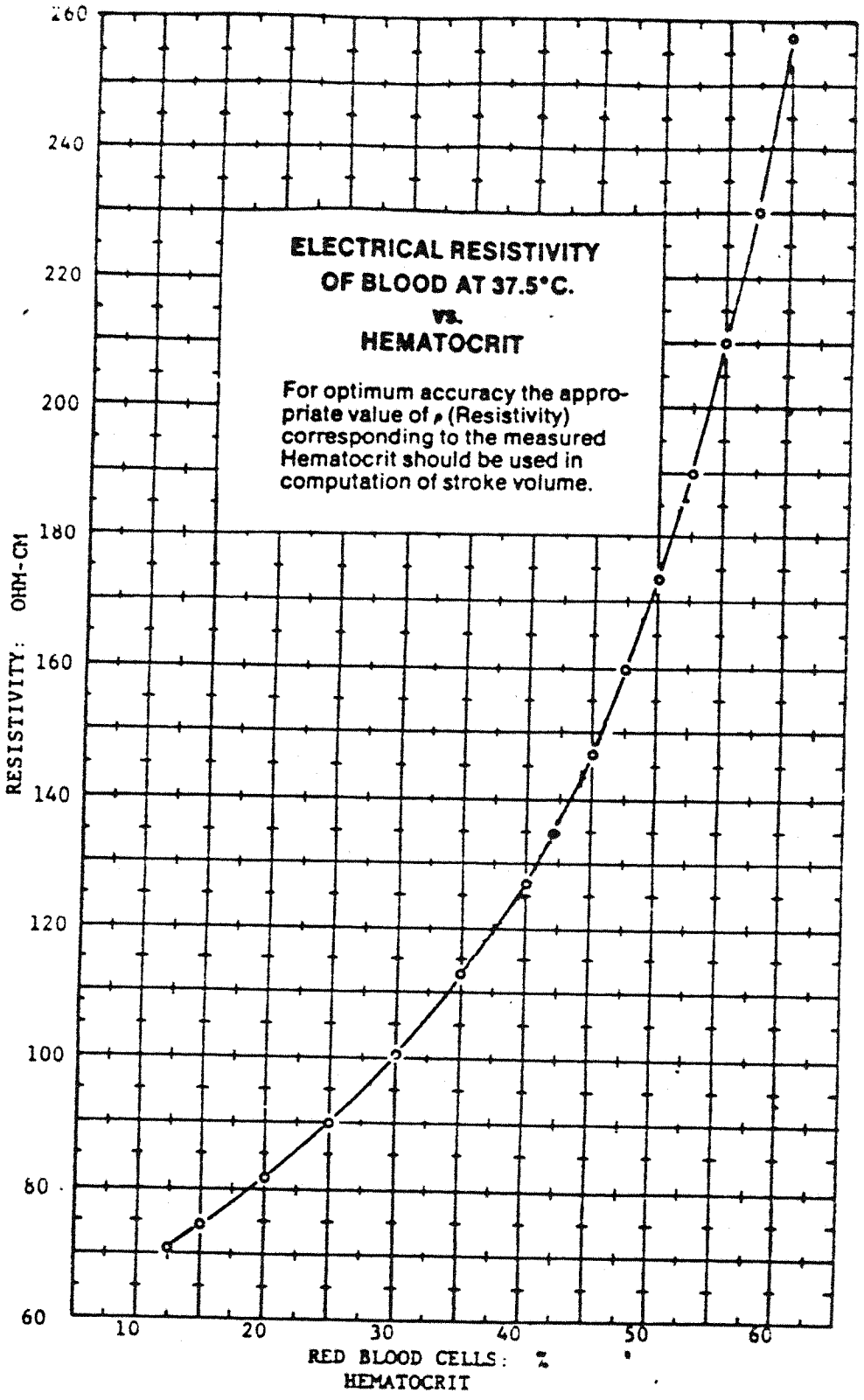


Fig. 1.7
The electrical resistivity of blood at 37.5 °C vs haematocrit value.

The cardiac output was calculated as HR x average SV. The arterial blood pressure was measured at the femoral artery with a pressure transducer (Elcomatic SL750) and recorded on a chart recorder (Kipp & Zonen BD8).

Peripheral conductance was calculated as cardiac output (CO) / mean arterial blood pressure (MAP) and therefore has the units of (ml/min)/mmHg. This term is the recipirocal of peripheral resistance and was used in preference for several reasons. The component of the expression most liable to error during measurement is the cardiac output, and conductance has cardiac output as the numerator rather than the denominator, as is the case with peripheral resistance. This keeps error due to measurement in this expression to a minimum. Conductances may also be easier to understand, being directly additive as determined by Ohm's law.

A Commodore PET microcomputer was used to calculate the results (SV, HR, CO, PC). Impedance cardiograph recordings were read into the microcomputer by a digitiser (Bit pad one, Summagraphics). The results were stored on magnetic disc for statistical analysis.

1.3.4 Nephrectomy.

Each rat was anaesthetised and prepared as in 1.3.1, then a midline abdominal incision was made. The kidneys were cleared of perirenal fat, the renal blood vessels were isolated and a ligature placed around them close to the kidney. Care was taken to ensure that the adrenal blood vessels were not occluded. In sham operated rats the ligatures were left untied. The incision was closed by suturing the peritoneum and then the skin. A period of 60 minutes was then allowed for stabilisation of cardiovascular function before the experiment was continued.

1.3.5 Statistical methods.

Animals were allocated to experimental groups using computer generated random numbers.

The results for each group were initially calculated as the mean $\pm$ the standard error of the mean (SEM) for each group, but most results were also calculated as percentage changes from pre-burn or pre-mock-burn values.

Differences between means were evaluated statistically by the Student's 't' test for paired or unpaired observations as appropriate.

1.4. RESULTS

1.4.1 Changes in cardiovascular function after burn or mock-burn injury and the effects of captopril administation.

24 rats were randomly allocated to 4 groups:-

Mock-burn	+	saline
Mock-burn	+	captopril
Burn	+	saline
Burn	+	captopril

Experimental measurements were started 60 minutes after the completion of preparative surgery. The row of dashes below represents the time scale of the experiments, from 30 minutes before to 90 minutes after the burn or mock-burn (which was applied at t=0 mins).

```
 x x        x  x          x          x          x
-30_________0_________+30_________+60_________+90
 *     *                  *          *
```

x = Cardiovascular measurements taken
* = Injection of 10ng angiotensin I

Measurements of stroke volume, heart rate, haematocrit etc were taken at -30, -25, -5, +5, +30, +60, +90 minutes, as indicated by x on the time scale above.

Thirty minutes before burn or mock burn injury the rats were pre-treated with either captopril (0.24 mg/Kg) or saline (0.9%) Both were administered as an I.V. bolus injection (0.5ml), over a period of 5 minutes. Bolus injections of angiotensin I (10ng in 0.2ml saline) were given at -30, -20, +45, +75 minutes, as shown by * above, to test the efficacy of the captopril. All animals were sacrificed after the experiment.

Fig.1.8 shows that before pre-treatment with captopril or saline there were no statistically significant differences between the mean cardiac output values of the four groups. Furthermore there was no significant difference in cardiac output between rats after treatment with saline or captopril.

The effectiveness of the captopril administered was verified by the measurement of the pressor response to angiotensin I. Saline pre-treatment did not significantly

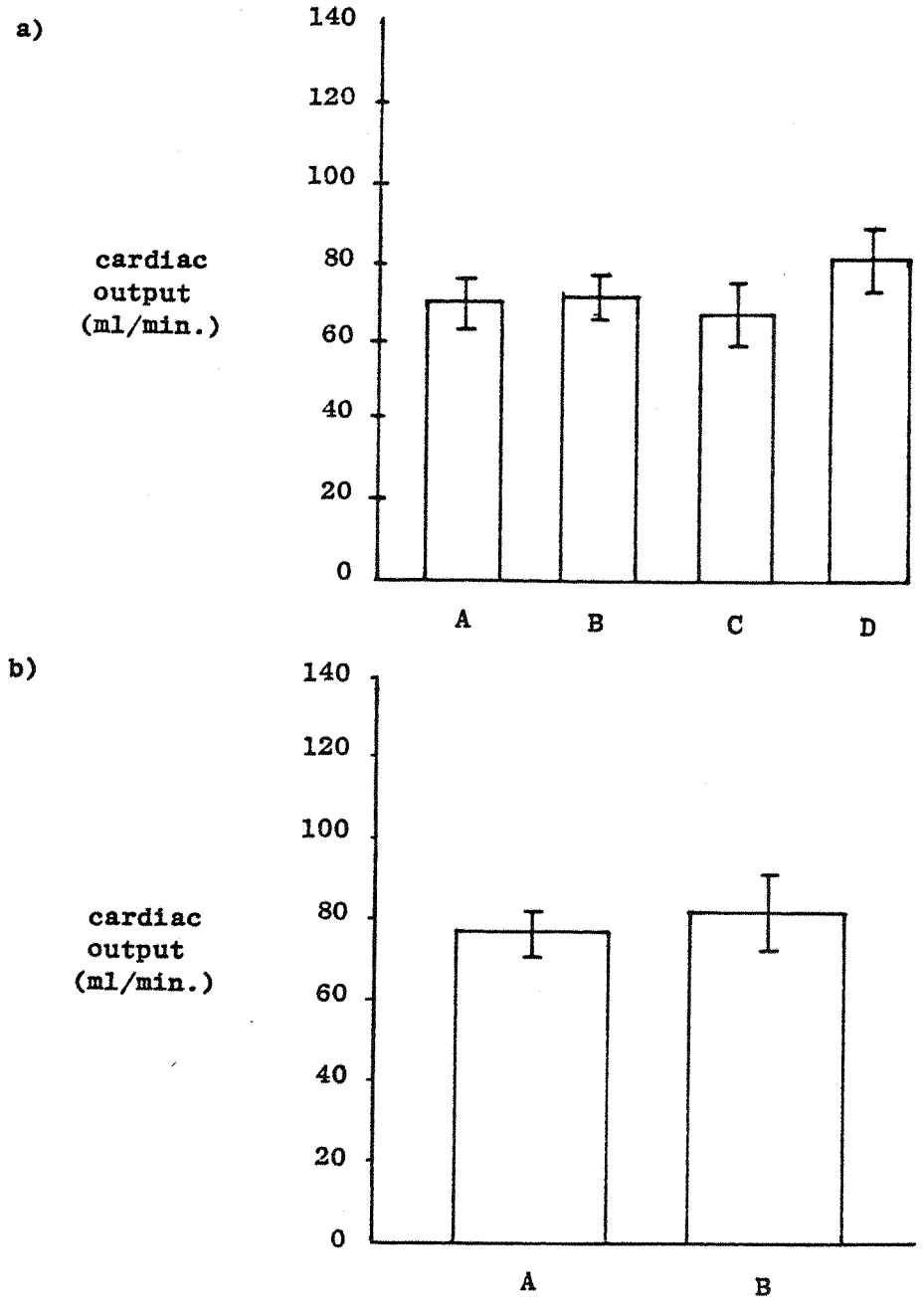


Fig. 1.8
Measurements of cardiac output;
a) before pretreatment,
A - Saline pre-treated, mock-burned rats, n=6.
B - Captopril pre-treated, mock-burned rats, n=6.
C - Saline pre-treated, burn injured rats, n=6.
D - Captopril pre-treated, burn injured rats, n=6.

b) after pretreatment with;
A - Saline, n=12
B - Captopril, n=12.

The values are shown as mean ± SEM and were evaluated statistically by paired Student's t-test.

change the blood pressure response to injected angiotensin I, while captopril pre-treatment decreased the pressor response from +11 ± 1 mmHg to +1.8 ± 0.6 mmHg ($P<0.001$, $n=6$).

Fig.1.9a shows that in saline pre-treated rats, burn injury caused a significant fall in cardiac output of 21 ± 6% ($P<0.05$) at 5 mins. post-burn. No decrease in cardiac output was seen in mock-burned saline pre-treated rats. In fact, after mock-burn the cardiac output increased by 11 ± 11% at 30 mins. post-mock-burn but was elevated by only 2.2 ± 14% at 90 mins. post-mock-burn; these small increases were not statistically significant.

Captopril pre-treatment however, greatly reduced the depression of cardiac output caused by burn injury (Fig.1.9b). After the mock-burn, cardiac output decreased slightly by 7 ± 6% at 5 mins. post-mock-burn and remained stable until 90 mins. post-mock-burn. In burn injured captopril pre-treated rats, the cardiac output was similarly decreased by 8 ± 8% at 30 mins. post-burn but then returned toward the pre-burn value at 90 mins. post-burn. None of these small decreases was statistically significant.

Cardiac output is the product of stroke volume and heart rate and the changes in these are shown in Figs 1.10 and 1.11. In saline pre-treated, burn injured rats, stroke volume decreased by 17.9 ± 7% at 5 mins. post-burn and 26 ± 7% by 90 mins. post-burn. This fall was statistically significant by 30 mins. post-burn ($P<0.05$). The stroke volume of the saline pre-treated, mock-burned rats, however, showed a slight non-significant increase until 30 mins. post-mock-burn, then fell to pre-mock-burn levels at 90 mins. Thus captopril pre-treatment abolished the decrease in stroke volume normally seen after burn injury (Fig.1.10b). The changes in stroke volume were therefore parallel with those in cardiac output.

Saline pre-treated rats showed no significant change in heart rate after mock-burn or burn injury. Captopril pre-treated rats, however, showed small (but statistically significant) decreases in heart rate after mock-burn or burn injury: the heart rate fell by 6.6 ± 1% ($P<0.05$) at 5 mins. post-burn and by 7.5 ± 2% ($P<0.01$) at 5 mins post-mock-burn. In both groups the heart rate remained at that level until 90 mins post-burn.

Fig. 1.12 shows the changes which occurred in arterial blood pressure. Rats which had been mock-burned showed no statistically significant change from pre-mock-burn levels irrespective of pre-treatment with

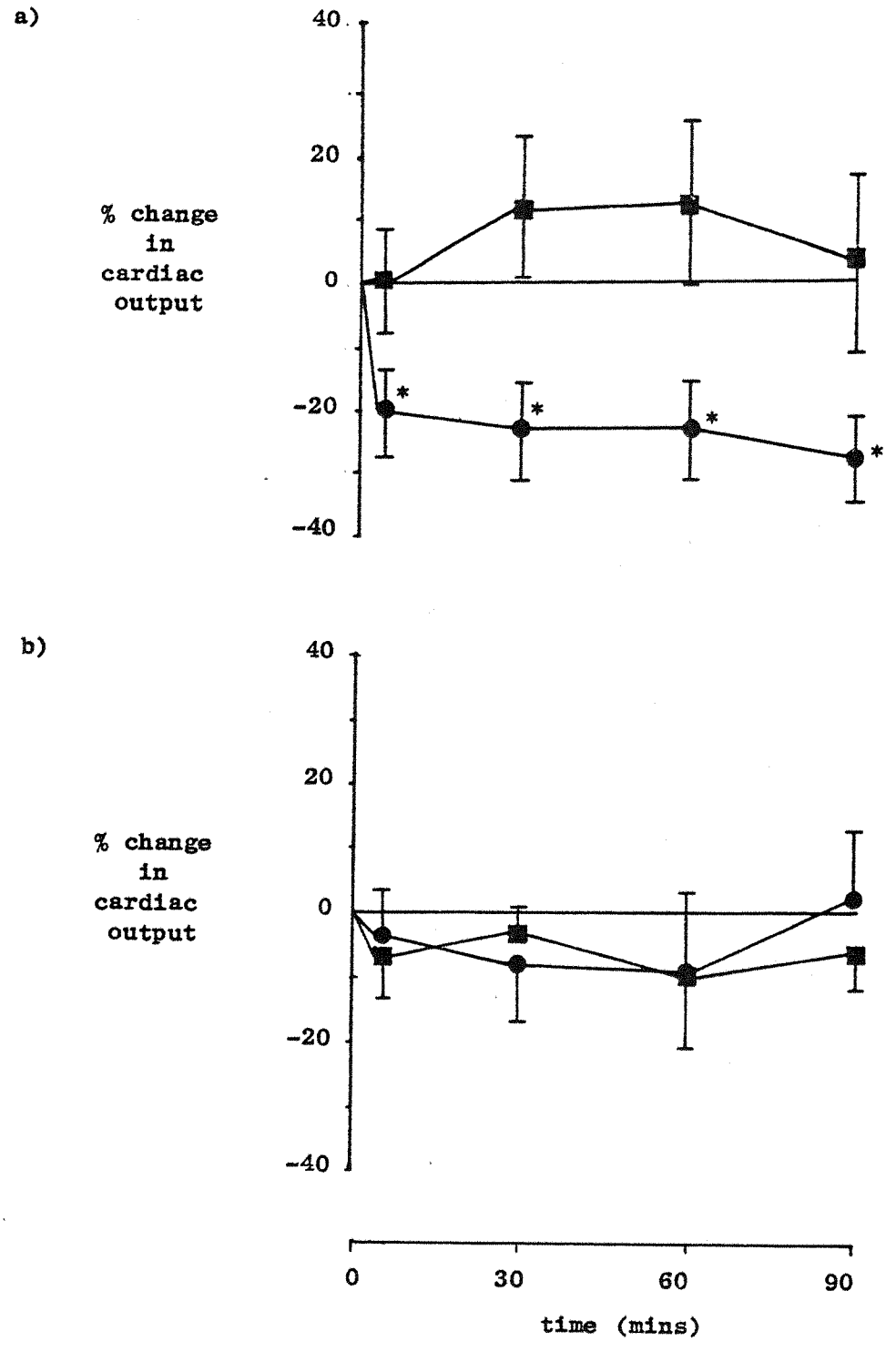


Fig. 1.9

The changes in cardiac output after burn and mock-burn injury;

a) after pre-treatment with saline,

b) after pre-treatment with captopril.

The values are shown as mean ± SEM (n=6 for all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05

** - P < 0.01

*** - P < 0.001

■ - mock-burned rats.

● - burn injured rats.

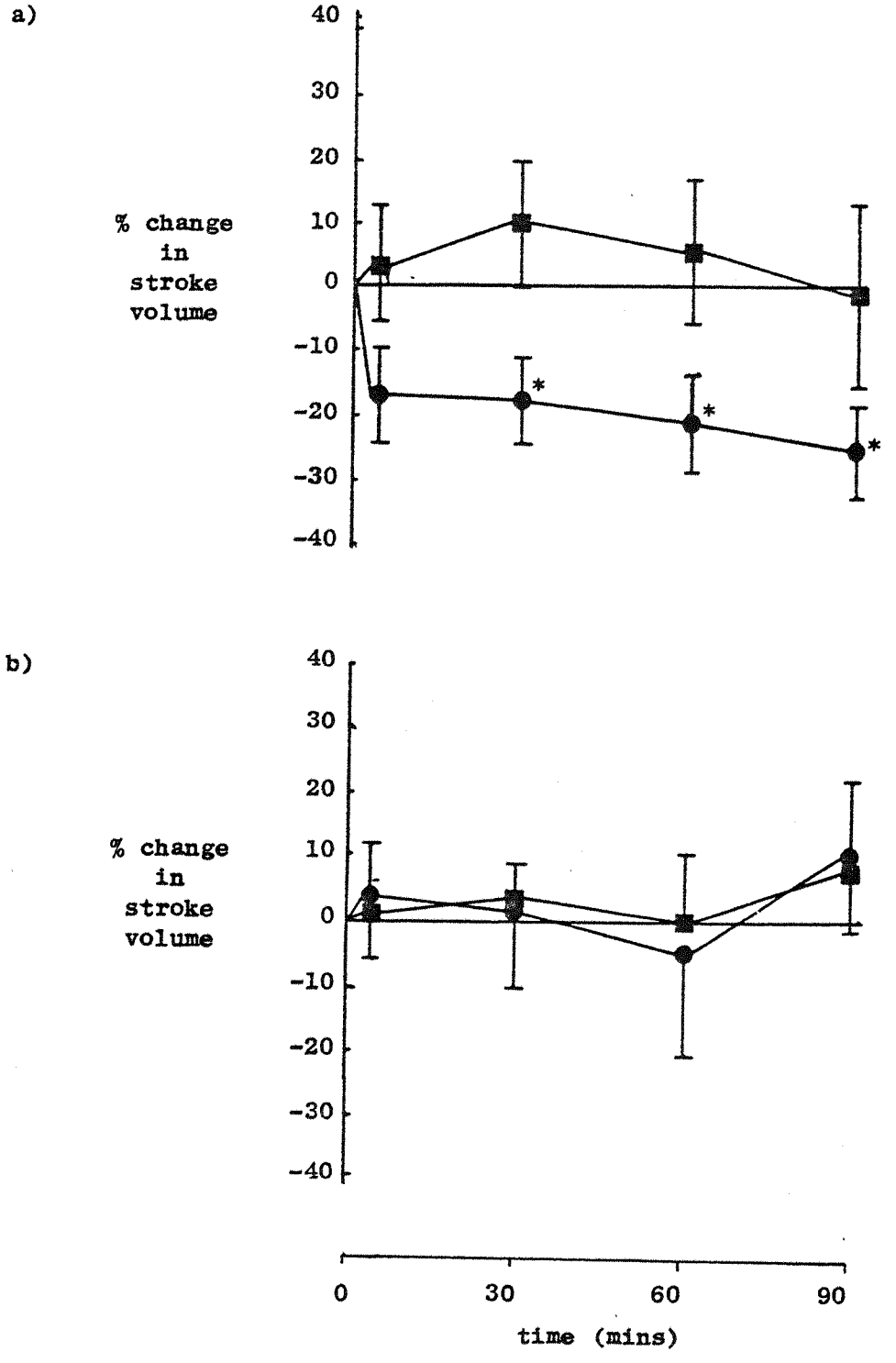


Fig. 1.10
The changes in stroke volume after burn and mock-burn injury;

a) after pre-treatment with saline,

b) after pre-treatment with captopril.

The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05
** - P < 0.01
*** - P < 0.001

■ - mock-burned rats.
● - burn injured rats.

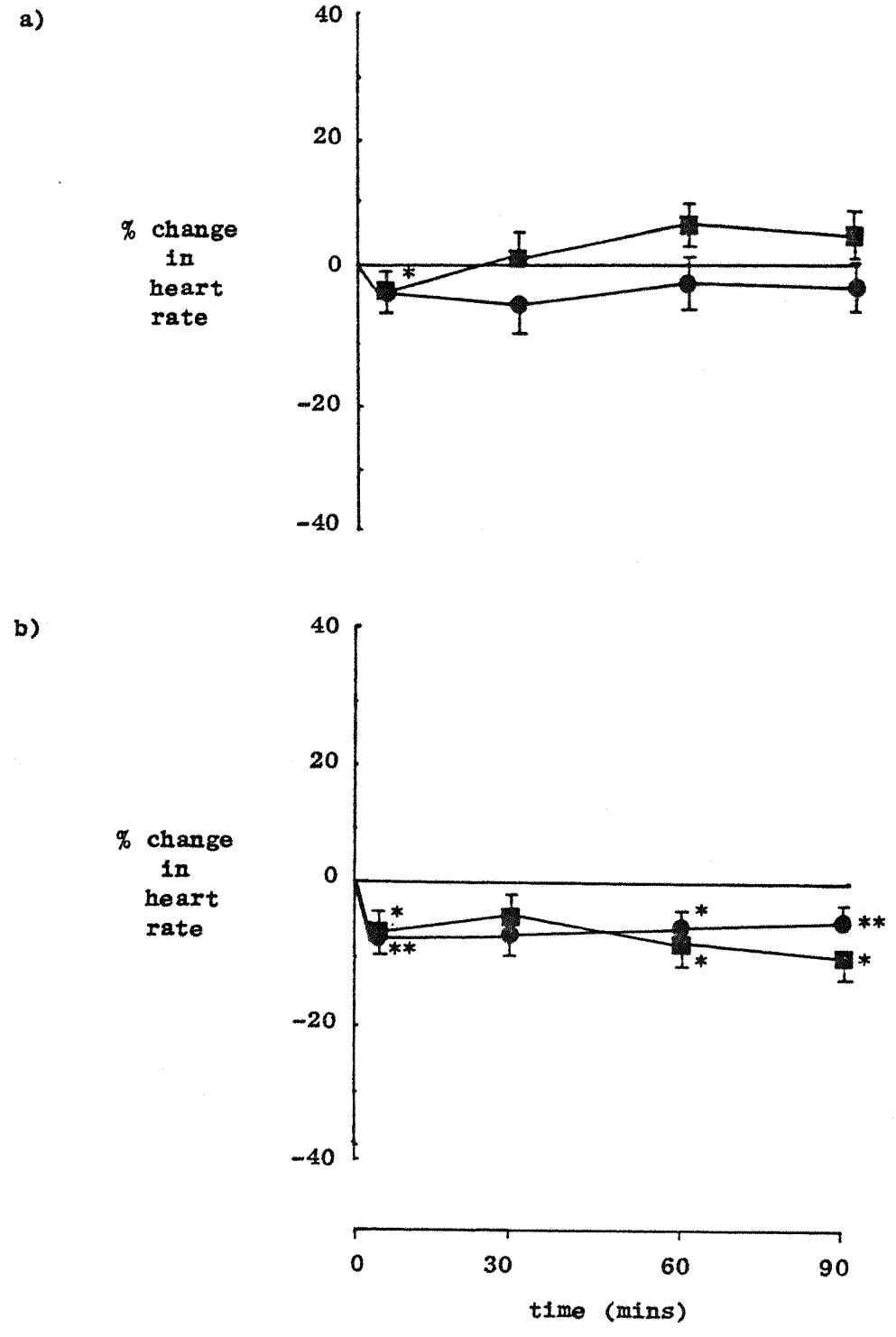


Fig. 1.11
The changes in heart rate after burn and mock-burn injury;
a) after pre-treatment with saline,
b) after pre-treatment with captopril.
The values are shown as mean ± SEM (n=6 in all groups).
Results were evaluated statistically by paired Student's t-test.

* - P < 0.05 ■ - mock-burned rats.
** - P < 0.01 ● - burn injured rats.
*** - P < 0.001

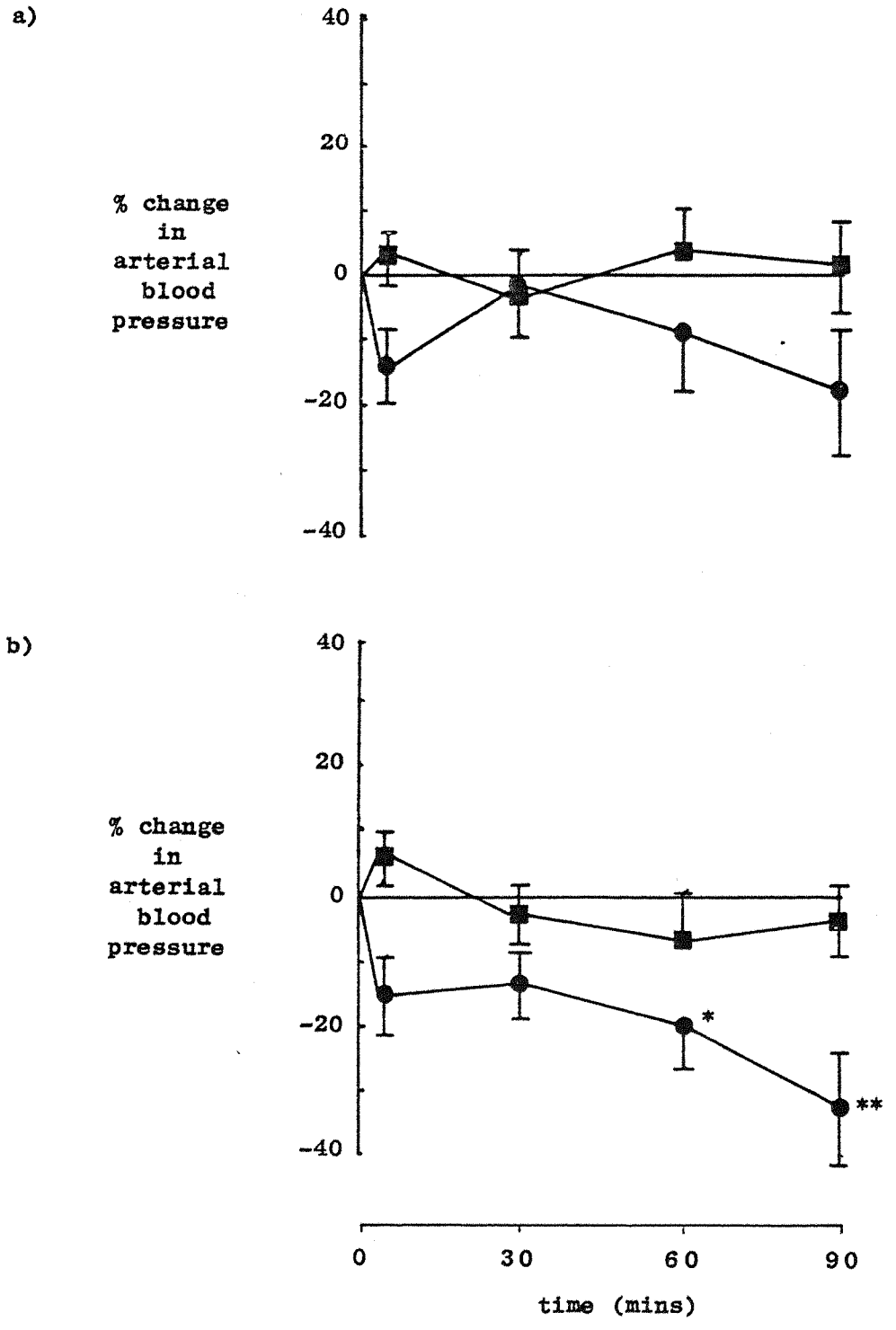


Fig. 1.12
The changes in arterial blood pressure after burn and mock-burn injury;
a) after pre-treatment with saline,
b) after pre-treatment with captopril.
The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05 ■ - mock-burned rats.
** - P < 0.01 ● - burn injured rats.
*** - P < 0.001

saline or captopril. All rats subjected to burn injury, however, showed decreases in blood pressure: the decrease was most marked in captopril pre-treated rats, where blood pressure fell by 14.8 ± 6% (NS) at 5 mins. post-burn, 19.8 ± 6% ($P<0.05$) at 60 mins. post-burn and 32.9 ± 7% ($P<0.01$) by 90 mins. post-burn. In saline pre-treated rats, burn injury caused a similar initial fall in blood pressure by 14.3 ± 5% at 5 mins. post-burn but the blood pressure recovered almost to pre-burn values ,viz -2.2 ± 7% at 30 mins. post-burn, then decreased steadily to -18.4 ± 8% by 90 mins. post-burn. None of these decreases in blood pressure, however, was statistically significant. Thus captopril did not prevent the initial fall in blood pressure after a burn injury but did tend to prevent the subsequent partial recovery.

Changes in peripheral vascular conductance to blood flow (CO/BP), are shown in Fig.1.13. In saline pre-treated rats ,the conductance rose slightly after mock-burn e.g. by 15.4 ± 13% ($P>0.05$) at 5 mins. post-mock-burn. Burn injury, however, caused a fall in conductance of 20.9 ± 8% at 30 mins. post-burn. The conductance then slowly returned towards control levels. Mock-burn injury caused very little change in conductance in captopril pre-treated rats. Burn injury, however, caused an early increase in conductance of 19.4 ± 18% after 5 mins. This effect gradually diminished until 60 mins post-burn. At the end of the experiment the conductance tended to increase greatly (by 68 ± 32%) although this change was not statistically significant.

Fig.1.14 shows the changes in haematocrit during this experiment. Mock-burn injury did not cause significant changes in haematocrit in either saline or captopril pre-treated rats. Burn injury produced very similar changes in saline and captopril pre-treated rats. There was an initial rise in haematocrit, which remained elevated throughout the experiment. The rise was slightly larger in captopril pre-treated rats: 7.5 ± 1% ($P<0.001$) at 30 mins. post-burn and 5.2 ± 1%, ($P<0.05$) at 30 mins. post-burn in the saline pre-treated rats.

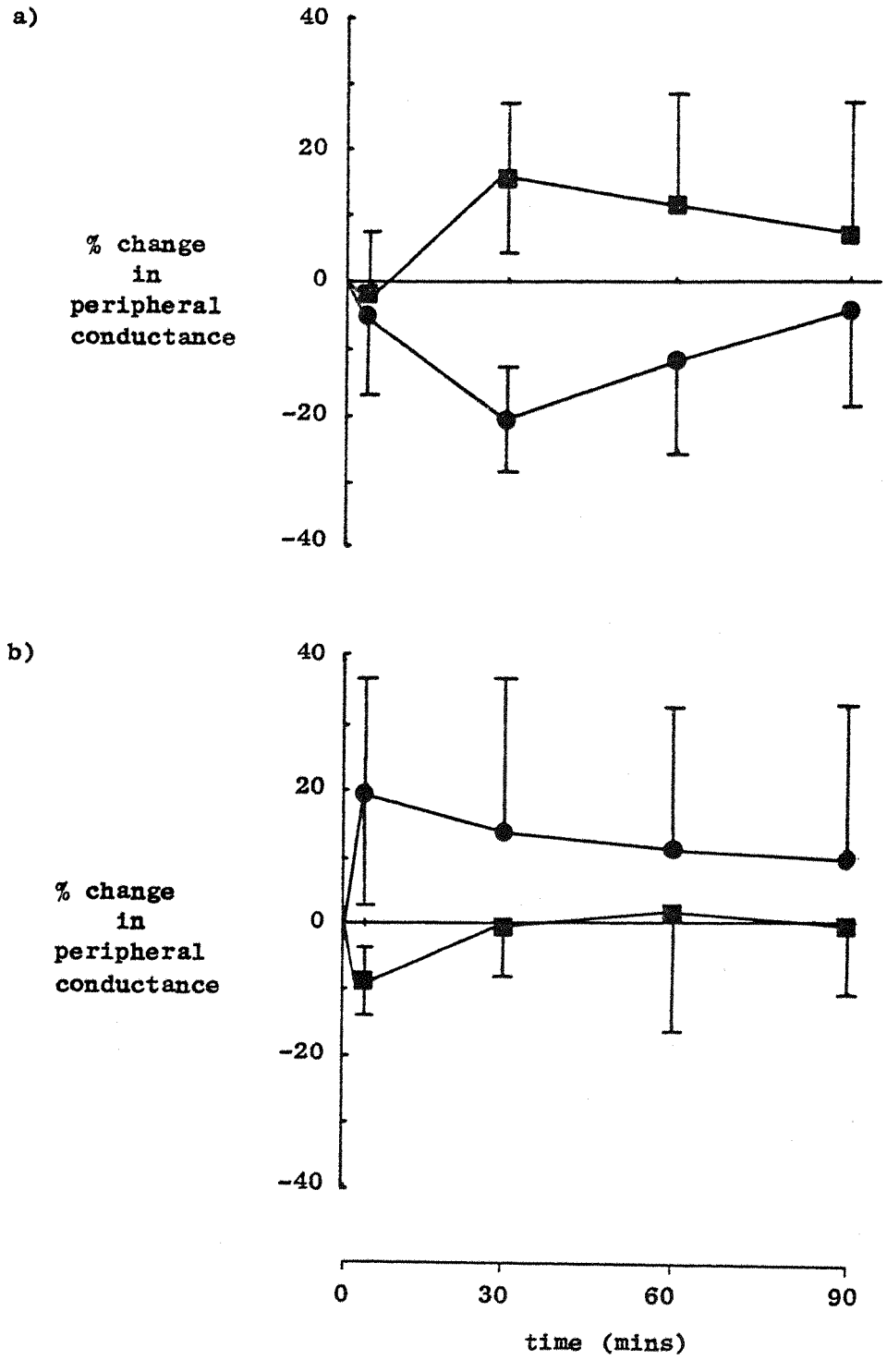


Fig. 1.13
The changes in conductance after burn and mock-burn injury;

a) after pretreatment with saline,
b) after pretreatment with captopril.

The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05
** - P < 0.01
*** - P < 0.001

■ - mock-burned rats.
● - burn injured rats.

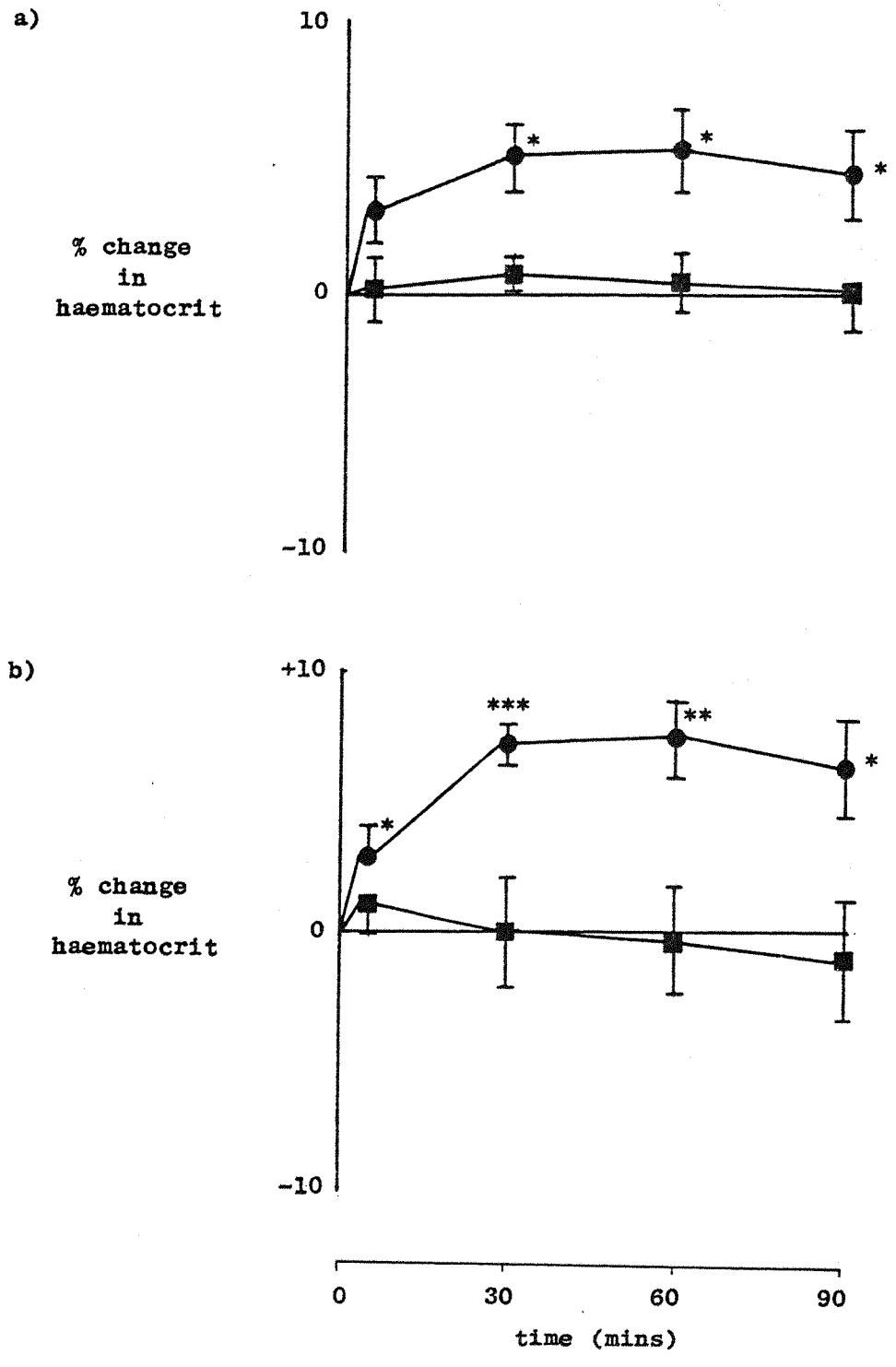


Fig. 1.14

The changes in haematocrit after burn and mock-burn injury;

a) after pre-treatment with saline,

b) after pre-treatment with captopril.

The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05

** - P < 0.01

*** - P < 0.001

■ - mock-burned rats.

● - burn injured rats.

1.4.2 The effect of saralasin on the cardiovascular function of burn and mock burn injured rats.

24 rats were randomly allocated to four groups:-

Mock Burn	+	saline
Mock Burn	+	saralasin
Burn	+	saline
Burn	+	saralasin

Cardiovascular measurements were taken at the times shown below (marked as x):-

```
x        x x        x          x    x    x          x
-30_______0_______+30_______+60_______+90_______+120
          ↑                   *    I----*----I     *
      burn or                      infusion of
      mock-burn                    saralasin
```

Burn or mock-burn injury was applied at t=0 mins. At 75 mins post-burn, an infusion of saralasin was started (5 mg kg^{-1} min^{-1} in 0.9% saline, at 0.9ml/hr). This infusion was stopped after 30 mins. A bolus injection of 50ng of angiotensin II (in 0.2 ml saline) was administered at 60, 90 and 120 mins after burn or mock-burn injury, as indicated by * on the time scale shown above.

Saralasin is reported to be an antagonist of angiotensin II action, and preliminary experiments were performed to demonstrate that the dose of saralasin selected for the present experiments was sufficient to block the pressor activity of angiotensin II. Fig.1.15 shows the pressor responses to various doses of angiotensin II in the presence and absence of saralasin (5mg kg^{-1} min^{-1}). In the presence of saralasin, a 32 times greater dose of angiotensin II was required to cause a pressor response of 30mmHg.

In this study the rats showed typical cardiovascular responses to burn injury or mock-burn injury: mock-burned rats showed a slight non-significant increase in cardiac output from 68.8 $\pm$ 4.6 ml/min before mock-burn to 75.6 $\pm$ 1.1 ml/min (NS, n=24) at 60 mins. post-mock-burn, and burn injured rats showed a fall in cardiac output from 67.8 $\pm$ 2.6 ml/min pre-burn to 52.4

± 2.8 ml/min (P<0.001, n=24) at 60 mins post-burn. There was also a significant decrease in vascular conductance in burn injured rats from 0.782 ± 0.046 (ml/min)/mmHg pre-burn to 0.656 ± 0.048 (ml/min)/mmHg at 60 mins post-burn (P<0.01 ,n=24). In mock-burned rats, however there was a slight increase in conductance from 0.774 ± 0.05 (ml/min)/mmHg to 0.822 ± 0.056 (ml/min)/mmHg (P>0.05, n=24). The changes in blood pressure, heart rate and stroke volume were similar to those in the previous experiment: burn injury reduced the mean arterial blood pressure from 91.5 ± 3.8 mmHg to 84.7 ± 3.5 mmHg and the stroke volume from 0.184 ± 0.006 ml to 0.148 ± 0.006 ml (P<0.001, n=24). The heart rate showed only a small non-significant change from 363 ± 8 to 355 ± 8 beats/min. Mock-burn injury caused no significant change in blood pressure, stroke volume or heart rate.

The infusion of saralasin into these burn injured rats caused two statistically significant changes. The cardiac output rose significantly after saralasin infusion from 53.0 ± 3.6 to 60.6 ± 4.6 ml/min post-infusion P<0.05,n=12), while saline infusion caused no change in the cardiac output, which was 53.8 ± 4.2 before and 53.6 ± 3.0 ml/min at 30 mins. post-infusion, (n=12). Saralasin infusion also caused a significant increase in the vascular conductance of burn injured rats, from 0.582 ± 0.064 to 0.746 ± 0.062 (ml/min)/mmHg (P<0.05,n=12); saline had no significant effect on the conductance, which was 0.730 ± 0.074 (ml/min)/mmHg before and 0.718 ± 0.034 (ml/min)/mmHg after saline infusion (NS, n=12).

Neither saline nor saralasin infusion caused any significant change in blood pressure, heart rate or stroke volume in these burn injured rats (although blood pressure tended to fall and heart rate and stroke volume to rise following the infusion of saralasin).

In the mock-burn injured rats, neither saline nor saralasin infusion caused any significant change in any of the cardiovascular functions measured.

In this experiment the activity of the infused saralasin was tested by administration of 50ng of angiotensin II in 0.2ml saline before and during infusion of saralasin or saline. Saralasin decreased the pressor response of the angiotensin II from +23.3 ± 3 mmHg to +2.1 ± 1 mmHg (P<0.001, n=12). Saline infusion caused no significant change in the pressor response to angiotensin II (+26.5 ± 2 mmHg to +23.6 ± 3mmHg, (NS, n=12)

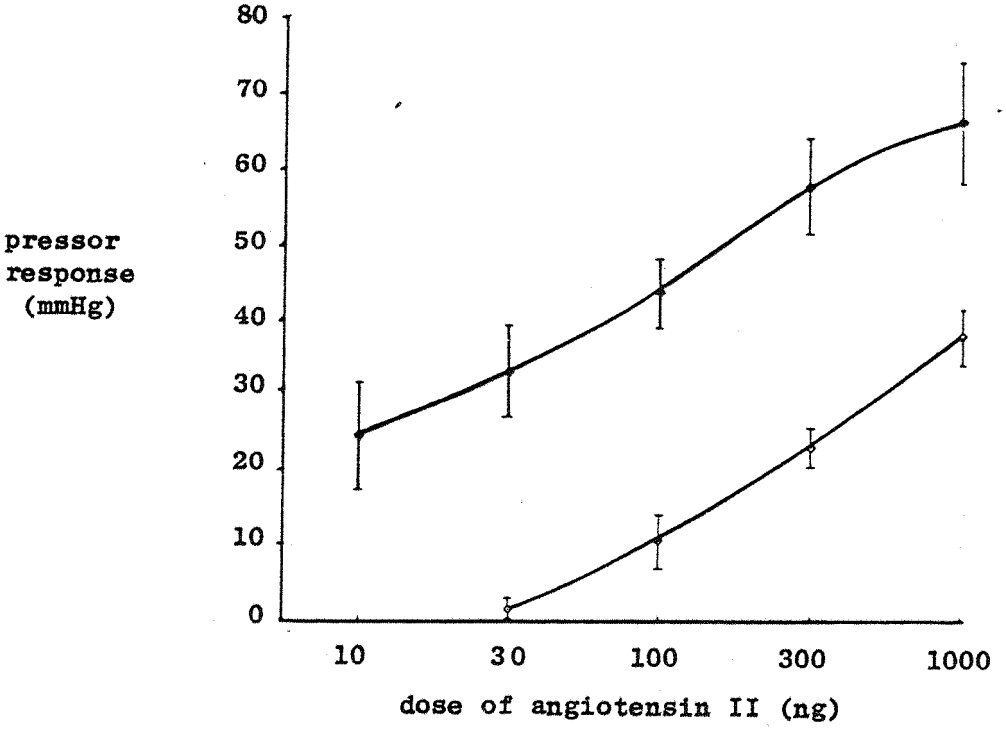


Fig. 1.15
The pressor activity of endogenous angiotensin II in the rat and the inhibitory effect of saralasin.

◆ - Angiotensin II alone.
◇ - Angiotensin II + saralasin.

1.5 DISCUSSION.

The control values of the cardiac output derived by impedance cardiography were comparable to those obtained by Chen (1981) and Philpot (1981) using impedance cardiography, thermodilution and electromagnetic flow transducer. This suggests that the impedance method can produce accurate measurements of cardiovascular function in the rat.

The application of a burn injury to an animal requires that the animal be handled, moved, partially immersed in water and that all measuring devices be disconnected and then re-connected. It is important to be certain that changes in cardiovascular function are due to the burn injury and not to the handling of the animals. For this reason mock-burn injured rats were taken as control animals.

Following mock-burn injury various small changes in cardiovascular function were observed, but these were generally not statistically significant. Cardiac output tended to increase slightly in most experiments, as did stroke volume. The heart rate, however, was generally unchanged, as was the blood pressure. Vascular conductance, therefore, also tended to increase slightly. The haematocrit did not change after mock-burn injury. It can therefore be concluded that mock-burn injury itself did not cause any important changes in cardiovascular function, thus any change observed in the burn injured group must be due to the burn injury rather than the associated handling etc.

Burn injury caused consistent changes in cardiovascular function. In all of the experiments there was a fall in cardiac output of approximately 20% by 5 minutes post-burn and this level was maintained until the end of the experiment (usually 90 minutes) as reported elsewhere. This decrease was associated with a concomitant decrease in stroke volume. The heart rate did not show consistent changes and the small changes that occurred were probably not great enough to influence cardiac output. The fall in cardiac output after burn injury was therefore due to the decrease in stroke volume not to changes in heart rate.

As discussed above, there are three factors which can be responsible for a fall in stroke volume: preload, afterload and myocardial contractility. In the present studies, central venous pressure (a measure of the preload of the heart) was not measured. Much evidence shows that burn injury leads to rapid oedema formation and hence

hypovolaemia, causing an elevated haematocrit (see section 1.2.2). In the present experiments the haematocrit consistently increased after burn injury, suggesting that blood volume and hence CVP would be reduced. However, the cardiac output was found to be significantly reduced within 5 minutes of burn injury even though the haematocrit had not significantly changed at that time. Furthermore, Aggarwal, (1983) using techniques similar to those used in these experiments, observed only a small non-significant reduction in CVP during a 30 minute period following burn injury in the rat. Also, Philpot et al,(1980) found that pre-treatment with cimetidine, a specific histamine receptor antagonist, prevented the change in haematocrit while the cardiac output still fell; similar results have been reported with other anti-inflammatory agents (Hilton, 1981). It is therefore probable that hypovolaemia (and therefore decreased preload) is not responsible for the immediate fall in cardiac output.

The present experiments were designed to test whether the renin-angiotensin system, (which is known to have marked effects on the afterload of the heart via its powerful actions on the peripheral vasculature and arterial blood pressure), is involved in the cardiovascular changes observed after burn injury. Two experiments were performed: cardiovascular function after burn injury was studied in animals treated with captopril, to prevent angiotensin II formation, or with saralasin, to prevent angiotensin II action. Tests were performed which confirmed that the dose of captopril used was effective in this respect ie. the pressor response to angiotensin I was abolished.

Captopril greatly reduced the depression of cardiac output characteristically seen after burn injury, by abolishing the fall in stroke volume. This improvement in cardiac output could be due to changes in the preload, afterload, contractility or heart rate.

Measurements of haematocrit indicate that captopril did not prevent the loss of plasma from the circulation after a burn injury; also it has been reported that angiotensin II does not alter the capacitance of the venous system (Bumpus & Page, 1974). Thus changes in angiotensin II production should not influence the preload on the heart. Inhibition of angiotensin II production is not the only effect of captopril. ACE (or kininase II) is also responsible for the breakdown of bradykinin, and an elevation of plasma bradykinin has been demonstrated after captopril administration (Marks et al, 1980). Endogenous

bradykinin has been shown to cause vasodilatation and increased capillary permeability (Laffan et al, 1978). In the experiment reported in this thesis, captopril pretreatment tended to enhance the elevation in haematocrit produced by burn injury and this observation could be explained by a captopril-induced elevation in bradykinin concentration (even though it has been reported that captopril may prevent the bradykinin-induced increase in capillary permeability in rat skin via a yet undefined mechanism (Fantone, Schrier & Weingarten, 1982). These observations suggest that captopril should not increase the preload on the heart and hence the improvement in cardiac output of burn injured rats caused by captopril is probably not due to an increased preload.

Captopril prevented the decrease in peripheral conductance usually seen in the early post-burn period, which suggests that this decrease in conductance is at least partly caused by angiotensin II.

The beneficial actions of captopril in haemorrhagically shocked cats have been described by Trachte & Lefer (1978). Captopril, however, elevated mean arterial blood pressure when given post-haemorrhage (as compared to sham treated control animals), indicating a prolongation of circulatory stability (which was closely related to survival). These workers observed that captopril caused decreases in: the circulating level of angiotensin II, total plasma proteolysis, circulating lysosomal hydrolase activity (as determined by cathepsin D activity) and circulating haemorrhagic shock myocardial depressant factor. These workers also reported that captopril has no direct inotropic effects on an in vitro papillary muscle preparation.

There is now mounting evidence that angiotensin II is involved in the facilitation of adrenergic neurotransmission and captopril has been shown to attenuate adrenergic vasoconstriction in the rat (Collis & Keddie, 1981). Captopril may therefore help to attenuate the vasoconstriction caused by burn injury in two ways: by preventing the direct action of angiotensin II on smooth muscle and by attenuating the increase in sympathetic vasoconstrictor tone. Indeed increased angiotensin II production could be the mechanism of the reflex vasoconstriction caused by the falling blood pressure, as reported by Shik & Len'kova, (1979) in anaesthetised rabbits.

In the second experiment, saralasin was used as an antagonist of angiotensin II, again to test the possible

involvement of the renin-angiotensin system in the cardiovascular response to burn injury. The dose of saralasin used was shown to block the pressor response to I.V. injections of angiotensin II. Saralasin has been shown to act as a partial agonist of angiotensin II (Pals et al, 1971), but no such action was evident in the present studies.

Saralasin was infused into burn injured rats at one hour post-burn and caused significant increases in cardiac output, peripheral vascular conductance and a decrease in blood pressure. The heart rate also increased after saralasin infusion so this may have contributed to the improvement in cardic output in burn injured rats. At one hour post-burn the infusion of saline in the control rats produced slight changes in cardiovascular function, probably due to increases in blood volume and preload on the heart, as seen during fluid resuscitation after burn injury. These changes were less than those seen in the saralasin infused rats. This experiment therefore showed that the effects of the saralasin infusion were similar to those of captopril with the exception that the improvement in cardiac output involved a change in heart rate as well as in stroke volume.

The results from these two experiments suggest that the renin-angiotensin system is involved in the depression of cardiac output in the early post-burn period. The results from this study suggest that after a severe burn injury, the renin-angiotensin system acts to preserve the arterial blood pressure at a time when the cardiac output is depressed, by causing an increase in peripheral resistance. When the renin-angiotensin system was blocked, the arterial blood pressure tended to fall after burn injury, thus leading to a fall in the afterload on the heart. This fall in afterload probably contributed to the improvement in stroke volume seen in these animals. However, an increase in afterload after burn injury does not simply explain the decrease in cardiac output, and similarly the fall in afterload in burn injured animals, where the renin-angiotensin system is inactivated, does not easily explain the improvement in stroke volume in these animals, as the heart is normally insensitive to increases in afterload.

The work of Cohn and Franciosa (1973), however, indicates that although the normal heart is relatively insensitive to afterload, a failing heart becomes very sensitive to afterload. The suggestion that heart function may be impaired after burn injury, is not new (Shoemaker, 1962; Merriam, 1962), but could help to

explain the findings of the present study. The concept that the 'burn shock' syndrome may be initiated by a deterioration in heart function has not been thoroughly investigated. The rest of this thesis describes studies on the relevant methodology and experiments performed to investigate this hypothesis further.

CHAPTER 2:
The development of a system for the measurement of left ventricular pressure in the rat.

2.1 INTRODUCTION.

In chapter one it was proposed that the early decrease in cardiac output in the burn injured rat could be due in part to an early deterioration in heart function. I therefore decided to investigate heart function in the early post-burn period directly. This presented several difficulties: studies on heart function in the rat have seldom been reported in the literature, probably because of the small size of the rat preparation and the short duration of the cardiac cycle in the rat. The choice was either to change the species for experimental study or to develop a rat preparation in which cardiac function could be studied. Since the Southampton University burns research group has used the rat model successfully for several years, the latter seemed more appropriate. It was preferable for the new model to be a closed chest model, so that comparisons could be made between this heart function preparation and the rat model used in previous studies. This limitation meant that the most appropriate way to assess cardiac performance in the rat model was to measure left ventricular pressure (LVP).

This chapter describes the problems encountered during the development of a heart function preparation. The main problems were in obtaining accurate measurements of left ventricular pressure but also minimising the disturbance caused to the cardiovascular system of the rat. In order to obtain the most accurate possible measurements of LVP it was necessary to measure and experiment with the frequency response characteristics of the pressure transducer system. A simple procedure called the drop test was devised to assess the frequency response characteristics of the pressure transducer system. Furthermore, in order to process the results mathematically, simple signal processing techniques were applied using a microcomputer.

2.2 LITERATURE REVIEW AND BACKGROUND INFORMATION.

2.2.1 Problems with the fluid filled catheter pressure transducer.

Fluid filled catheter pressure transducer systems are commonly used for the measurement of arterial and venous blood pressures in small animals. The usual type of pressure transducer has a metal diaphragm which is linked to an internal strain gauge, which is displaced by a column of fluid.

For such systems to reproduce fluctuating pressure signals accurately, the electrical response must occur reliably and rapidly. A change in blood pressure forces a small volume of fluid along the catheter to deform the transducer membrane and this deformation is transduced into an electrical signal. The membrane deformation and electrical signal are proportional to the pressure applied. There is, however, resistance to the flow of fluid within the system and this resistance must be kept to a minimum to allow the system to respond rapidly. The catheter may be slightly elastic and the fluid within the system may be compressible, hence more fluid needs to move than is actually required to deform the transducer membrane, and this further limits the ability of the system to respond rapidly. The components which move (i.e fluid and membrane) have inertia : it takes a finite time to set them moving and they continue to move beyond the equilibrium point for any applied pressure. This gives rise to resonance within the system, i.e. the transducer membrane and the fluid, tend to oscillate. The resistance to flow encountered by the fluid results in a decrease in the velocity of pressure transmission through the system, causing damping of a fluctuating signal and also introduces a time delay. This time delay may be seen as a phase difference between the actual pressure and the measured pressure. Consequently, measurements of pressure may be modified by the measuring system itself. Distortion of a pressure recording becomes more likely as the frequency of the applied pressure rises.

Thus, two factors influence the degree of distortion introduced to a pressure recording by such measuring systems: resonance and damping. Like all structures pressure transducers vibrate in response to an oscillating input signal, and the amplitude of this vibration varies with the frequency of the input signal. The frequency of the input signal causing maximum vibration of a structure is called the natural or resonant

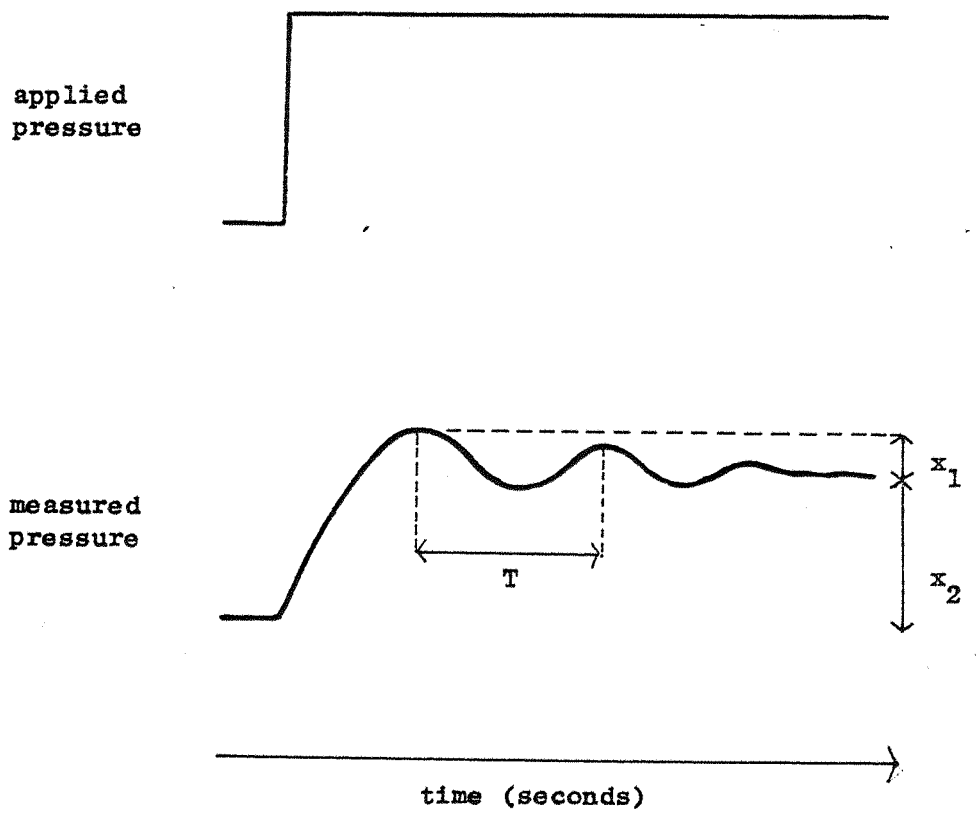
frequency. The exact relationship between the frequency of the input signal and the amplitude of the vibration induced is discussed in detail later.

In most situations encountered in the physiology laboratory, pressure measurements fluctuate relatively slowly or with too small an amplitude for such distortion to be significant. In the barbiturate anaesthetised rat, where the heart rate is 400 beats per minute and peak left ventricular systolic pressure is 100 mmHg, the left ventricular pressure may rise by 100mmHg in 5-10 milliseconds. In this case significant errors may be introduced into the measured pressure signal by the resonance and damping characteristics of the measuring system. These errors are then magnified when the LVP is differentiated to give dP/dt, the peak value of which is a commonly used index of left ventricular contractile function.

2.2.2 The mathematical modelling of pressure transduction systems.

Pressure transduction systems generally behave in the same way as a simple damped oscillator or second order system (Fry, 1960). The effects of resonance and damping on such systems can be understood by considering the response of a damped oscillator to a sudden displacement (Fig.2.1). As the coefficient of damping is increased (an increase in the damping within the system), the measured response changes in form, so that the oscillations are reduced in magnitude and die away more rapidly. When the coefficient of damping reaches 1, a condition known as critical damping, the system responds to a displacement in a simple exponential manner. A system with a coefficient of damping < 1 is said to be underdamped, while a system with a coefficient > 1 produces a response which is overdamped and only slowly drifts towards the maximum amplitude of the original signal (Fig.2.2).

This model can predict the variations in amplitude and phase which occur with different frequencies of input signal i.e. the distortion imposed on the measured signal by the transducer system. The way in which a measuring system alters an input signal is summarised in a Bode plot. This consists of two graphs which show the variation of amplitude with frequency and also phase difference with frequency. The Bode plot completely describes how such a system will alter any input signal. The Bode plot for a second order system can be calculated by Eqns.(1) and (2):



T = time for 1 oscillation

f_d = damped natural frequency (1/T Hz)

h = coefficient of damping

$$h = \sqrt{\frac{(\ln x_2/x_1)^2}{\pi^2 + (\ln x_2/x_1)^2}}$$

Fig. 2.1
The response of a second order measuring system to a step in pressure.

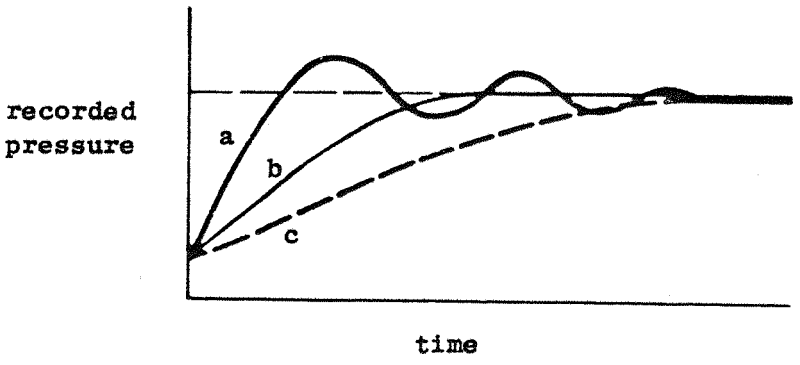


Fig. 2.2
The response of a second order pressure measurement system to a pressure step, and the effect of variation of the coefficient of damping (h):

a) $h > 1$ (underdamping),
b) $h = 1$ (critical damping),
c) $h < 1$ (overdamping).

$$(1)\quad \frac{\text{Amplitude in } (A_i)}{\text{Amplitude out } (A_o)} = \frac{1}{4h^2 B^2 + (1-B^2)^2}$$

$$(2)\quad \text{Phase angle } (\phi) = \tan^{-1} \frac{(2hB)}{1-B^2}$$

where h = Coefficient of damping

$B = \dfrac{\text{Input frequency } (W_0)}{\text{Natural frequency } (W_n)}$

Fig.2.3 shows the Bode plot for a damped oscillator system and how it varies for an underdamped, overdamped and critically damped system. The mathematical model can be extended to take into account the hydrodynamic and structural aspects of a fluid filled catheter pressure transduction system, as shown in Eqns. (3) & (4).

$$(3)\quad F_n = \frac{K_1 \cdot D_c}{V_d \cdot L_c \cdot h}$$

$$(4)\quad h = \frac{K_2 \cdot V}{D_c^3} \cdot \frac{L_c \cdot V_d}{D}$$

where F_n = Natural frequency (Hz).
h = Coefficient of damping.
D_c = Diameter of catheter (cm).
L_c = Length of catheter (cm).
V = Viscosity of fluid (Poise).
D = Density of fluid (g/cm^3).
V_d = Volume displacement coefficient for the system ($mm^3/100mmHg$).
$K_1 = 1.35 \times 10^{-3}$
$K_2 = 1.40 \times 10^{3}$

In particular, these relationships predict how the behaviour of such a system will vary with catheter length and diameter. These relationships are investigated further in this chapter. These equations assume that the transducer system acts exactly as a second order system with no complicating factors. There are several ways to discover if a transducer system is acting as a

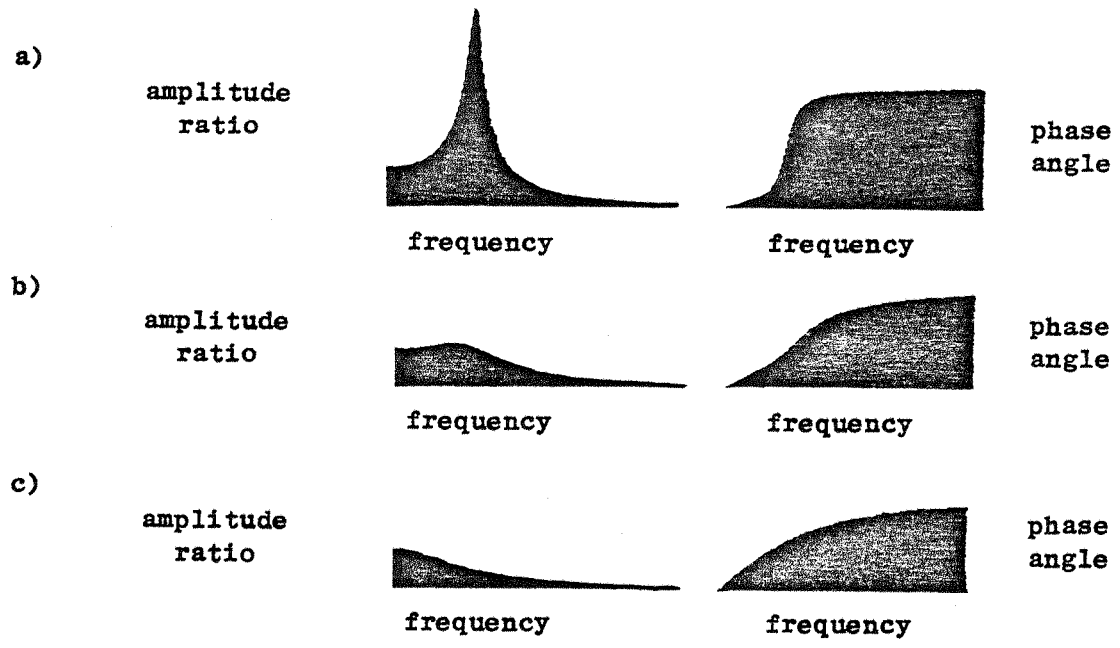


Fig. 2.3
The effect of variation of the coefficient of damping (h) on the amplitude vs frequency relationship of a second order pressure measurement system:

a) h > 1 (underdamping),
b) h = 1 (critical damping),
c) h < 1 (overdamping).

simple second order system as is discussed later.

2.2.3 The experimental determination of the frequency response characteristics of a pressure transducer.

There are two methods described in the literature for the experimental determination of transducer response characteristics: pressure step techniques (Wood, 1950; Shapiro & Krovetz 1970) and oscillation chamber techniques (Noble, 1959; Stegall, 1967).

a) An instantaneous step in pressure can be easily produced by bursting an inflated balloon, which has been secured over the tip of a fluid-filled catheter. The coefficient of damping and damped natural frequency of the transducer can be measured from the resultant pressure trace (Fig.2.1). The natural frequency of the transducer system can then be calculated from the damped natural frequency. This technique has the disadvantage that popping the balloon may physically vibrate the transducer. It is also difficult to ascertain the exact moment that the pressure step occurs. This information is essential for estimation of the time delay introduced by the system. Also, the pop-test produces useful information only if the system behaves as a simple second order system.

b) Oscillation chambers can be used to measure the response of the transducer to vibration at one discrete frequency (i.e. a sine wave). The cannula tip is placed in a fluid filled chamber and oscillations are produced in the fluid by a piston or an underwater loudspeaker. The frequency of the driving oscillations can be altered and the subsequent values of amplitude ratio and phase angle compiled to form a Bode plot for the system. This method has the disadvantage that sophisticated, expensive equipment is required.

The pop-test has been used in the work described in this thesis but in addition a new technique, the drop test, was devised to overcome some of the disadvantages of the two methods described above.

2.2.4 The use of Fourier analysis for the determination of frequency response characteristics.

It is well established that periodic (repeating in a cyclical manner), or transient (non-repeating) signals can be represented by a series of component sine and cosine waves (Fig.2.4). The simplest periodic wave, a sine wave can be mathematically defined by Eqn.(5):

(5) $\text{Amplitude } (A) = A_{max} \cdot \sin(wt)$

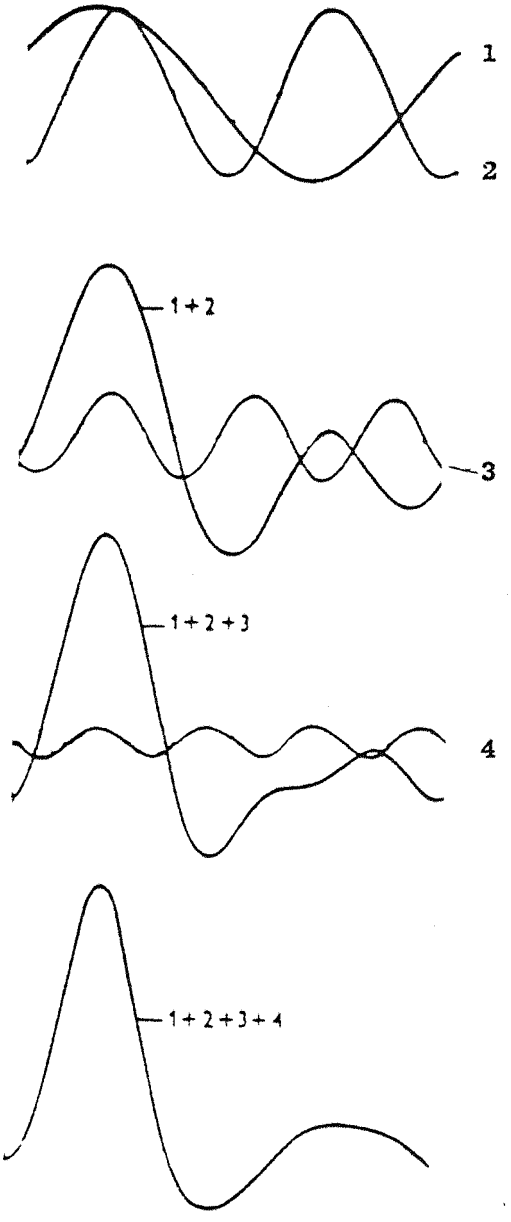


Fig. 2.4
The theoretical basis of Fourier analysis. Any periodic signal may be recreated by the addition of its component sine and cosine waves. This example of the recreation of a femoral artery pulse has been redrawn from Cobbold (1972).

where W = angular frequency (radians/second).
t = time (seconds).
A_{max} = maximum amplitude.

In the same way a complex periodic waveform can be represented by the sum of a series of sine and cosine waves (Eqn 6).

$$(6) \quad A = 1/2\ A_o + \sum_{n=1}^{n=\infty} (a_n \text{ Cos } nWt + b_n \text{ Sin } nWt)$$

Where:

A = The amplitude at time t.
A_o = The average amplitude of the waveform.
a_n = The cosine coefficient relating to component n.
b_n = The sine coefficient relating to component n.
t = Time elapsed in seconds.
n = Component number.
nW = Multiples of the fundamental frequency W.

Equation (6) shows that the amplitude of a waveform can be calculated at any time during its periodic cycle by adding half of the average amplitude (to allow signals to have an offset from zero) and the sum of a series of sine and cosine waves. This series of sine and cosine waves is called a Fourier series and the coefficients (a_n & b_n) are calculated by eqns. (7) & (8):

$$(7) \quad a_n = 2/T \int_0^T A(t) \text{ Cos } (nWt) \, . \, dt$$

$$(8) \quad b_n = 2/T \int_0^T A(t) \text{ Sin } (nWt) \, . \, dt$$

Where T = The period of the waveform in seconds

The Fourier series for a periodic waveform is calculated as follows. The amplitude of the waveform at known time intervals throughout one cycle of the waveform is A(t0), A(t1), A(t2)...A(T). To calculate the coefficients (a_n & b_n) of one component sine wave of frequency nW, every value of A(t) is multiplied by sin (nWt), i.e the recorded waveform is multiplied by a sine wave of frequency nW. If this sine wave is a large

component of the recorded waveform, the product of this multiplication will be increased and will always be positive (or negative if the waveforms are 180° out of phase). The products resulting from these multiplications are then summed and averaged by multiplication with 2/T. Any frequency other than nW will have approximately equal positive and negative products which will cancel out when summed together, leaving an & bn as a true representation of the magnitude of the contribution of sine wave nW to the recorded waveform.

The Fourier series produces a sine and a cosine coefficient to describe the contribution of each wave to the recorded signal (Eqn.6). Each component of the series can also be represented as a phase-shifted sine wave as shown in Eqn.(9).

(9) $\text{Ampl.}(A) = (a_n 2 + b_n 2) \cdot \text{Sin} (nWt - \phi)$

Where ϕ (Phase Shift) = $\tan\text{-}1 (a_n / b_n)$

The appearance of the cosine coefficient (a_n) in Eqn.(5) is thus the way in which phase shifts in the component waves are described.

In principle an infinite number of sine and cosine waves is required to completely describe a recorded waveform ($n=\infty$), but, in practice only a relatively small number is required to describe most biological signals (Attinger, Anne & Macdonald, 1963).

In principle, a transient signal cannot be represented by a Fourier series since it is not continuous but a finite event in time. The same principle can, however, be applied to transient signals to produce a spectrum of the different frequency components occurring in the signal.

Transient signals are analysed by discrete Fourier transformation, a process which depends on an auto-correlation analysis, as shown in Eqn(10):-

(10) $$Y = 1/T \int_0^T A(t)\, A(t-\tau) \cdot dt$$

where Y = Coefficient of auto-correlation.
τ = Time of previous sample in seconds.
T = The number of samples comprising the transient.
A = Amplitude of transient signal.

Every sample of the transient signal is multiplied by the value occurring τ seconds earlier. Generally there

will be no similarity between two points in a signal τ seconds apart and so the sum of these multiplications will tend to zero. If the transient signal does contain a component sine wave with a period of τ seconds, then $A(t).A(t-\tau)$ will generally be positive and therefore the sum of $A(t).A(t-\tau)$ will be non-zero. The sum of these products is then averaged (divided by T) to produce an auto-correlation coefficient. A set of these coefficients can be calculated for all possible values of τ and a plot of auto-correlation coefficient vs the period of the sine wave (τ), is analagous to a Fourier series. A Fourier transform (DFT) of Y produces a series of sine and cosine coefficients (a & b) for each frequency between 0Hz and the maximum possible frequency ($2/\tau$) in steps of (Fs /T) Hz. Discrete Fourier transformation is a lengthy, time consuming process, requiring N^2 multiplications and additions for a waveform composed of N sampled points, analysing the frequency content up to (Fs/2) Hz.

Until recently, such signal processing techniques could only be performed with the aid of large computers. The development of the fast Fourier transformation (FFT) has been a major advance, greatly facilitating the application of this signal processing technique. The FFT stores each of the coefficients (a & b) as a single complex number as shown in Eqn.(11):

(11) $\cos X = (e^{ix} + e^{-ix})$

$\sin X = (e^{ix} - e^{-ix})$

where $-i = \sqrt{-1}$

In the studies reported in this thesis, 512 data points were recorded at a sampling frequency of 4000Hz. The FFT was performed on a microcomputer with limited memory capacity (32Kbytes) and each transformation required 4608 computations instead of the 262144 computations required for a DFT.

In summary, FFT is a way of processing a recorded periodic or transient signal so as to obtain a series of sine waves which can reproduce that signal.

The FFT is used by vibration engineers to measure the frequency response characteristics of vibrating systems. They make use of the fact that an impulse of very short duration (such as a blow from a hammer), can set an impacted surface vibrating, such that the resulting vibrations in the impacted surface are called the impulse

response of the structure and are dependent on the characteristics of the surface itself. The response to the hammer blow is mathematically defined for a simple second order system by the impulse response function (see Eqn. 12):

(12) $A(t) = K\, e^{-hWnt} \,.\, \mathrm{Sin}\,(hWnt)$

Where :

A(t) = Amplitude of vibration at time t.
Wn = Natural Frequency of Structure (radians/sec).
h = Coefficient of Damping.
K = A constant relating to average signal amplitude.

It follows that the frequency response characteristics of a system can be measured by FFT analysis of the impulse response to a sudden brief blow which acts in the manner descibed above. This principle has been adapted for the measurement of the frequency response characteristics of fluid-filled pressure transducer systems and is described in detail in the methods section below.

2.2.5 The correction of errors induced by fluid-filled catheter pressure transducers.

Several methods have been devised to correct for (or avoid) the resonance and damping errors in physiological pressure measurements:

a) Catheter-tip manometer.

Catheter-tip manometers (i.e. where the pressure sensitive diaphragm is sited in the tip of the catheter), eliminate problems due to resonance. A catheter-tipped manometer with a maximum diameter of 1mm has been used to obtain high fidelity measurements of left ventricular pressure in the rat (Zimmer, 1984). Such catheters have frequency response characteristics which are claimed to be linear up to 25kHz and are therefore capable of recording biological signals with great accuracy. The disadvantage is the high cost of the catheter and its relative fragility (Zimmer, 1984).

b) Rational damping.

An underdamped transducer system causes resonance and phase changes in a recorded signal. These changes can be reduced by increasing the coefficient of damping for the system towards 0.707, the value at which the system is rationally damped, i.e the state of damping which allows the measured signal to stabilise at the level of the input signal in the shortest possible time. This can be achieved

by a screw valve placed in the fluid path or by increasing the viscosity of the fluid. The disadvantage of increasing the coefficient of damping is that the damped natural frequency of the system will be decreased as shown in Eqn.(13), causing a deterioration in the frequency range.

$$(13) \qquad W_d = W_n \sqrt{1-h^2}$$

where W_d = Damped natural frequency (radians/second)
W_n = Natural frequency (radians/second)
h = Coefficient of damping.

c) Deconvolution.

The tendency of the measuring system to generate damped oscillations can be quantified (in principle) by application of an instantaneous 'standardised pressure pulse' to the measuring system e.g. a pulse which rises momentarily then returns to the baseline value. The resultant oscillations in the pressure then define the basic resonance and damping characteristics of the measuring system. From such data we could calculate how the recording system would respond to any change in applied pressure. The mathematical process used to calculate this imposition of the measuring system characteristics onto the recorded signal is called convolution. The inverse process (i.e. removal of the systems characteristics from a recorded signal), is called deconvolution; this signal processing technique has been applied to the problem of fluid filled pressure transducer systems (Attinger, Anne and McDonald, 1960; Krovetz, Jennings and Goldbloom, 1974).

Deconvolution can be conveniently performed using FFT. The Fourier transform of a recorded pressure waveform is divided by the Fourier transform of the impulse response of the measuring system. Inverse FFT then reconstitutes a pressure waveform, which theoretically, should be free of the distortion introduced by the measuring system.

d) Electronic filters.

Another method for correction of pressure signals involves the use of an electronic filter to deconvolve recorded signals. The filter is constructed to allow the settings of natural frequency and coefficient of damping to be altered externally. The filter characteristics are determined by the mathematical model of a second order system (Eqn. 3), (Noble & Barnett, 1967; Falsetti, Mates, Carrol, Gupta & Bell, 1974). The great advantage of this method is that the deconvolution is done 'on-line' and the

results are available immediately. The obvious disadvantage is that the pressure transducer must behave exactly as a second order system if the resultant waveform is to be accurate, and this need not always be the case (Krovetz et al, 1974; Melbin & Spohr, 1974). Electronic devices which are filters modelled on higher order systems have been constructed but have met with limited success (Melbin & Spohr, 1974).

2.3 METHODS.

2.3.1 The assessment of transducer frequency response.

a) The measurement of pressure.

The pressure transducer used for these studies (an Elcomatic E750A) was a diaphragm-displacement type, with a single-crystal silicon diaphragm. For a detailed discussion of the mechanics of pressure transducers see Cobbold (1974). The transducers alone possess excellent frequency response characteristics , which are claimed by the manufacturer to be satisfactory up to 25kHz (when the catheter and dome are not fitted).

In the present studies, the transducer system consisted of the transducer and transparent perspex dome onto which two three-way taps were fitted. One tap was used to open the system to atmospheric pressure during calibration The other tap was connected to a shortened hypodermic needle (internal diameter = 0.8mm), which was in turn connected to a length of catheter tubing. The system was flushed with a wetting agent (ethanol) and carefully filled with 0.9% saline, which had been boiled in a vacuum for 30 minutes to remove dissolved gas. The internal fluid volume of the dome, without catheter attached was 0.52ml. The volume displacement coefficient for the system was estimated as 0.005 ml/100mmHg.

The transducer was connected to a Wheatstone bridge circuit whose output was amplified by a purpose built, low noise pre-amplifier. The output from this was fed to a microcomputer interface (Unilab 532-001), which digitised the pressure signal at a sampling rate of 4kHz with 8-bit resolution (i.e. the A-D converter could resolve a 200mmHg pressure range into 256 evenly spaced intervals, thereby giving a resolution of 0.78mmHg). The data were then passed to a BBC model B microcomputer.

The data were analysed by FFT and plotted as a Bode plot.

b) Assessment of Fn, Fd and h by the 'pop' test.

The transducer was prepared as described above, with the addition of 90mm of nylon catheter tubing (Portex 800/110/140) and mounted as shown in Fig.2.5. The catheter was secured to the pop test apparatus by an air-tight Tuohy-Borst connector (BD-3099, Beckton Dickinson Co.). A finger cot was attached to the apparatus, secured by a rubber O-ring and inflated to a pressure of approximately 60mmHg using a sphygmomanometer

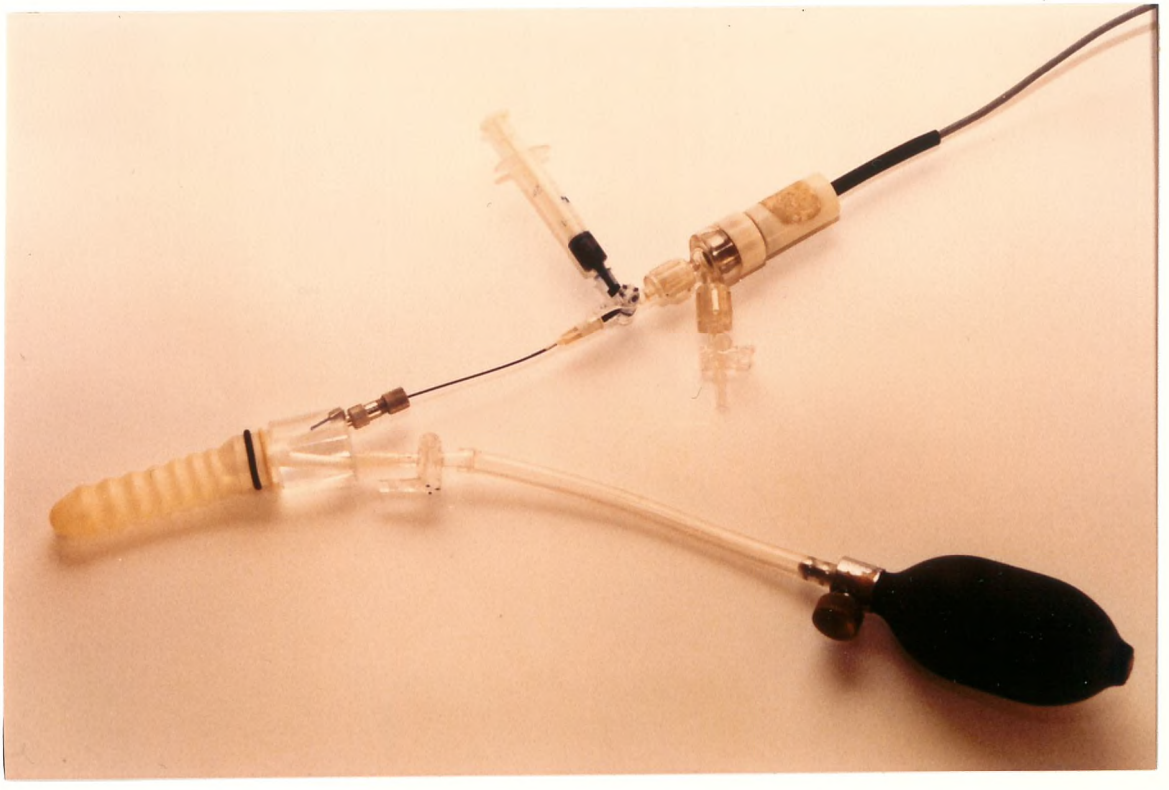


Fig. 2.5
The apparatus used for the pop-test.

bulb. The finger cot was then burst by a lighted match. The catheter tip was thus exposed to an instantaneous drop in pressure of around 60mmHg. The recorded pressure signal was stored in the microcomputer then displayed graphically as a plot of pressure vs time. Values for Fd and h were calculated by the method shown in Fig 2.1. The method described for calculation of Fn assumes that the transducer system acts as a second order system.

c) Assessment of the transducer frequency response characteristics by the 'drop' test.

The drop test generates an impulse response by applying a pressure impulse of short duration to the tip of the fluid filled catheter. In theory the impulse should be of infinitely brief duration, but in practice need only for the duration of one data sample (i.e. if the sampling frequency is 4000 Hz, the impulse must last for no longer than 0.5 milliseconds).

The pressure impulse was generated by allowing a drop of water to fall 30cm onto the upward pointing tip of the catheter (Fig.2.6). The exact time of arrival of the drop at the catheter tip was registered by an optically activated switch (Radiospares 304-560). The switch consists of a Gallium Arsenide, infra-red emitting diode, whose light output is directed towards a photodiode. The input and output of the photodiode were passed via a differential amplifier to a Schmitt trigger circuit. Breaking the infra-red beam switched the output voltage of the Schmitt trigger from 9V to 0V. The manufacturer claims that the time taken for the output to fall is between 25 - 50 nanoseconds. The catheter tip was placed directly below the beam and the output of the Schmitt trigger was fed to the microcomputer.

When the infra-red beam was broken by the falling drop of fluid, the fall in the output voltage from the Schmitt trigger was used to initiate the storage of the pressure measurements by the microcomputer. The first 512 sampled points of the resultant pressure waveform were stored in the microcomputer. Six of these pressure waveforms were averaged and the average was analysed by FFT and the resultant frequency response data were stored on disc.

d) The effects of using different catheters on the frequency-response characteristics of the system.

Catheters of different structure, length and internal diameter were studied by the drop test. The catheters were made of nylon, polythene or

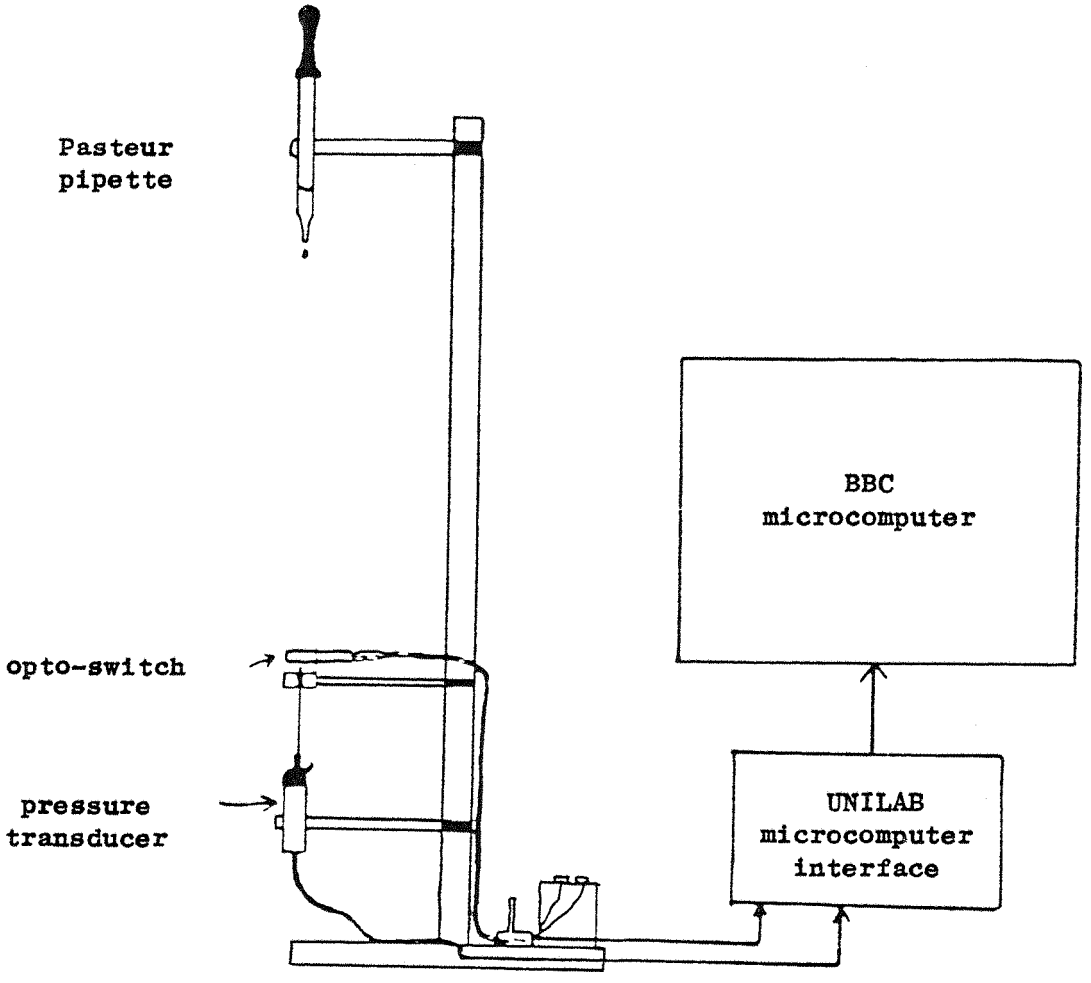


Fig. 2.6
The apparatus used for the drop-test.

polytetrafluoroethylene (PTFE).

2.3.2 The measurement and analysis of the left ventricular pressure in the anaesthetised rat.

a) Measurement of left ventricular pressure in the anaesthetised rat.

Female Wistar albino rats weighing 300-350g were anaesthetised with pentobarbitone (60 mg/kg I.P.). The rats were tracheostomised and kept at 37°C by adjusting the proximity of an anglepoise lamp. The drop-test procedure was used to record the frequency response characteristics of the transducer and catheter system (90mm of portex 800/100/175). The catheter was then inserted into the right common carotid artery and advanced via the aorta and aortic valve into the left ventricle. The presence of the catheter tip in the ventricle was verified by a diastolic pressure of approximately 0mmHg.

Left ventricular pressure waves were recorded in such a way that each trace started near the end of diastole, just prior to the onset of ventricular contraction, and lasted for 128 milliseconds. This time interval was long enough to capture ventricular contraction and ejection.

The intra-thoracic pressure was simultaneously recorded using a silicone rubber, oesophageal tube connected to a pressure transducer. Left ventricular traces recorded during periods of fluctuating intra-thoracic pressure were immediately rejected.

b) The frequency content of the LVP waveform.

The recorded portion of the left ventricular pressure waveform was analysed by FFT. The frequency content was described in two ways: as the variation in amplitude ratio with frequency (from the Bode plot), and as distribution of energy (i.e cumulative values of amplitude ratio) within the frequency spectrum.

2.3.3 The correction of left ventricular pressure waveforms.

a)The downsampling of pressure waveforms.

All pressure waveforms were recorded at 4kHz but it was considered helpful to reduce the amount of sampled data from 512 to 128 measurements i.e. by taking every fourth point and discarding the intermediate points. This procedure reduced the amount of data and effectively downsampled by a factor of 4 (i.e. sampling frequency

reduced from 4kHz to 1kHz). Unfortunately this procedure could introduce errors into the signal by a mechanism known as aliasing (see Cobbold, 1974 p34-65). In order to prevent this, the waveforms were passed through an anti-aliasing filter. This was a low-pass digital filter with a passband edge frequency of 400Hz and a stopband edge frequency of 500Hz (the stopband frequency was 45dB down on the passband frequency). In this way any frequencies greater than half the new sampling frequency were eliminated.

b) Deconvolution by manipulation of FFT data.

Deconvolution is achieved by division of the LVP FFT data by the system response FFT data. This is performed by the division of the complex numbers in which the FFT data is held. The notation for complex numbers is given in Eqn. (9). The equation for the division of complex numbers is given in Eqn. (14):

$$(14) \qquad \frac{a + ib}{c + id} = \frac{ac + bd}{c - d} + i \cdot \frac{bc - ad}{c - d}$$

Where a + ib = FFT data from system response
c + id = FFT data from LVP waveform

The result of this division is a deconvolved LVP waveform. This waveform sometimes contains high frequency noise which can be exaggerated by deconvolution, therefore the waveform is filtered by setting the high frequency components of the resultant FFT data to zero. The waveform is then reconstituted by inverse FFT.

2.3.4 Differentiation of the corrected LVP waveform.

Three methods of waveform differentiation were used:

i) Manipulation of FFT data - differentiation was achieved by the convolution (multiplication in the frequency domain) of the LVP waveform with a purpose built waveform. This waveform has an amplitude ratio which rises at 6dB and a time series which can be described by the following series: +1, -1, 0, 0, 0, 0, etc.

ii) Regression analysis - differentials were calculated using a 3rd order polynomial least squares regression analysis on 11 sequential data points, which calculated the slope at the 6th of the 11 points. This procedure also smoothed the data since the slope refers to the best fit for the 11 points

analysed. The equation below was used, where P-1, P, P+1, etc are used to represent the position of a value within the frame of 11 points.

Slope at point P = A - B - C - D - E

where: A = 0.05827 * (P-5 - P+5)
B = 0.05711 * (P-4 - P+4)
C = 0.10334 * (P-3 - P+3)
D = 0.09770 * (P-2 - P+2)
E = 0.05749 * (P-1 - P+1)

P-5 to P+5 are the 11 points necessary to calculate the slope at P.

iii) Analogue differentiation by an electronic circuit.

2.4 RESULTS.

2.4.1 The assessment of the frequency response characteristics of the pressure measuring system.

a) The 'pop' test.

A typical response to the pop test is shown in Fig.2.7. The balloon was inflated to a pressure of 60mmHg and then burst with a lighted match. In general, reproducible results were not obtained using the pop test because of the vibration caused by the bursting of the balloon. From theoretical considerations, the first differential of the time response from a pop test should be the same as the response to a drop test, (see section 2.2.3). Fig.2.8 shows the differentiated transform of a pop test. This transform is similar in appearance to that of the drop test.

b) The 'drop' test.

A typical time response to the drop test is shown in Fig.2.9a. This waveform is the average of 6 successive responses. The transform of this average drop test is shown in Fig.2.9b. The drop test was found to be extremely reproducible once the dropper had been correctly placed.

c) A comparison of system characteristics using the 'drop' and 'pop' tests.

The same catheter transducer system was subjected to both the pop and the drop test. The drop test data were analysed by FFT and the pop-test data were analysed graphically. The resonant frequency and coefficient of damping for the measuring system were:

Test	Fn	h
Pop-test	234 Hz	0.082
Drop-test	234 Hz	0.077

d) The assessment of the drop test as a true impulse response.

It is necessary to consider the possibility that the falling water droplet may be acting on the fluid in the catheter tip for too long, so that the impulse given to the pressure measuring system lasts for longer than one recorded pressure sample i.e longer than 0.1 msec. For technical reasons, this was impossible to measure directly, therefore, this had to be assessed indirectly using two different methods. FFT of a true impulse

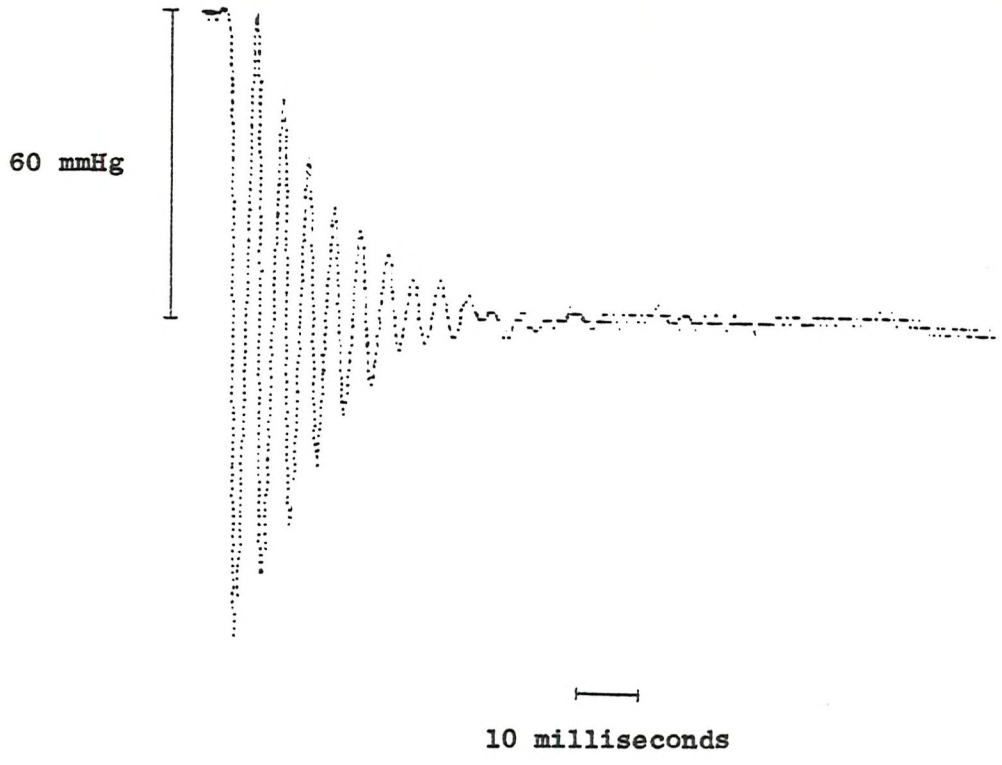


Fig. 2.7
A typical time response for the pop-test.

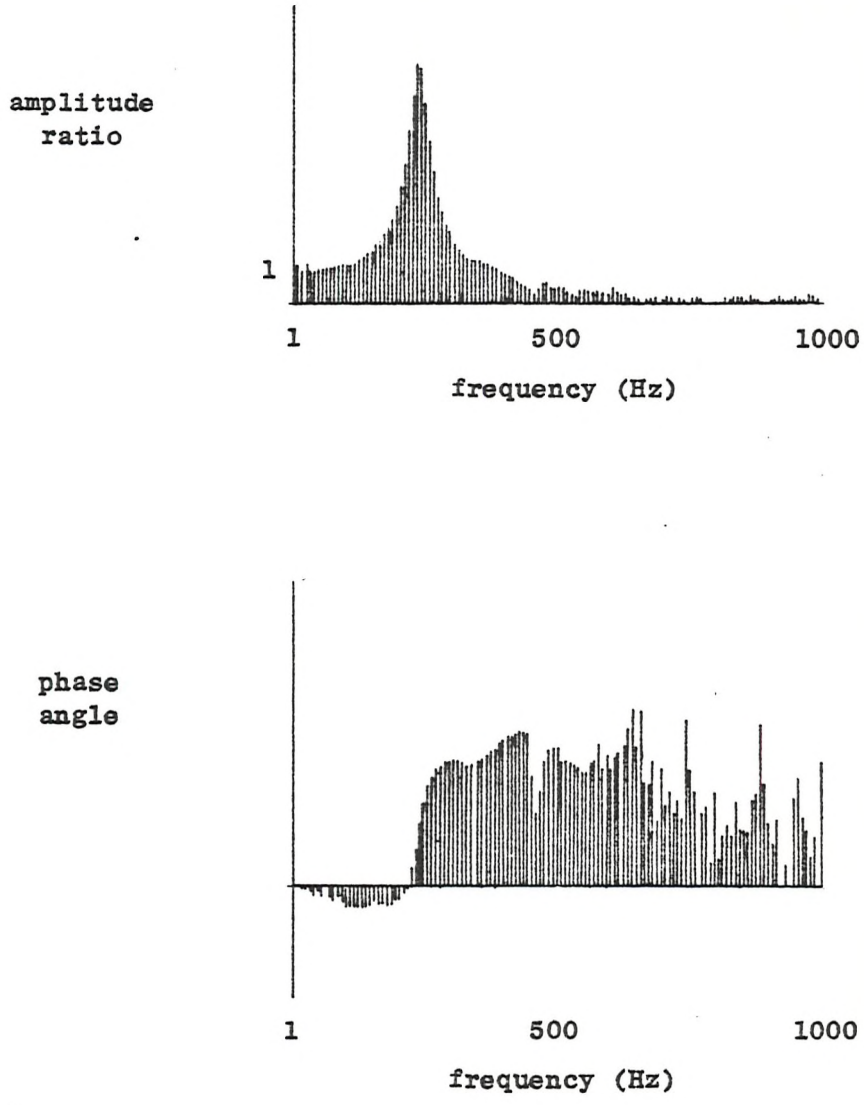


Fig. 2.8
The Bode plot of a differentiated pop-test.

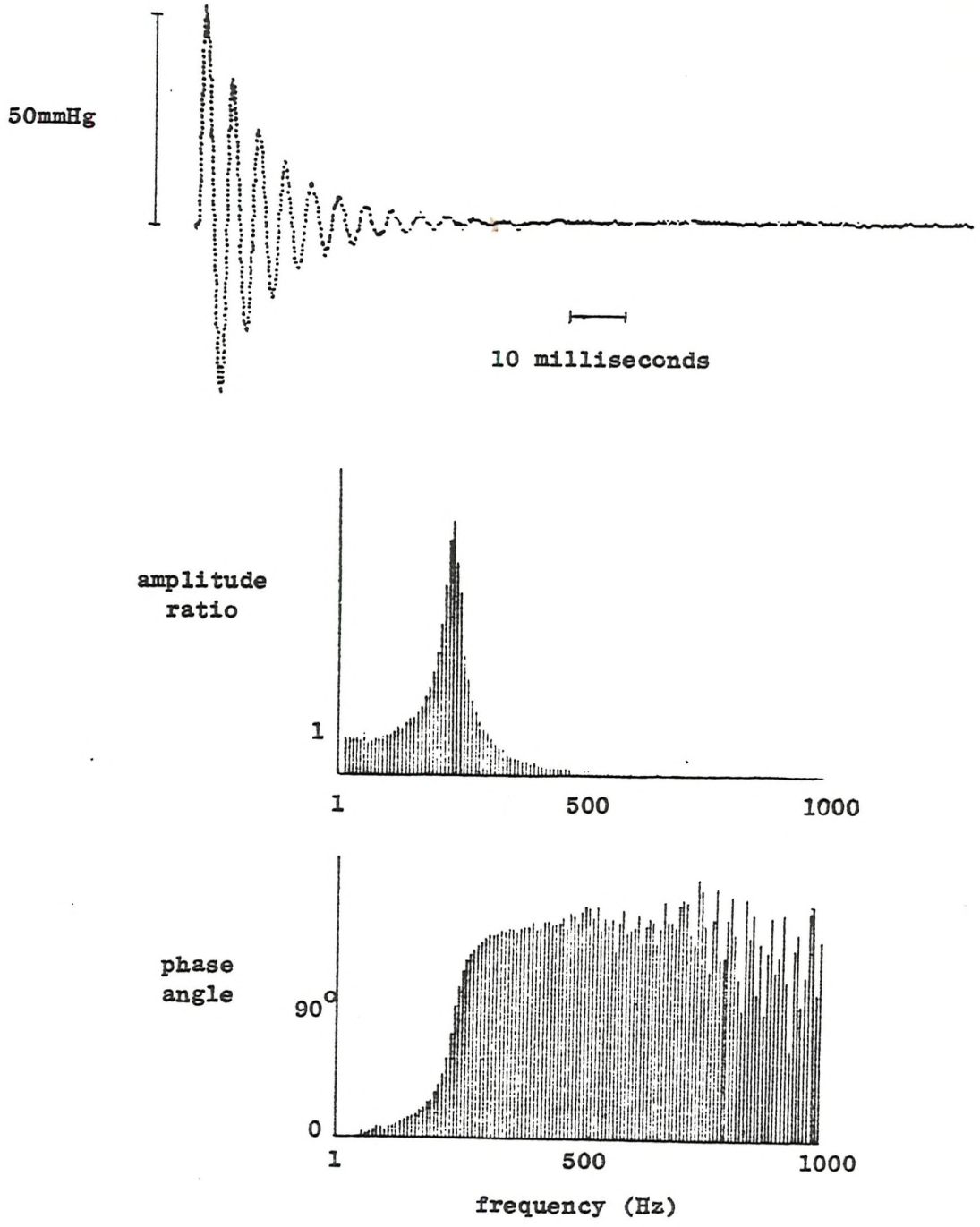


Fig. 2.9
The response of the pressure measurement system to the drop-test:

a) The time response (this waveform is an average of 6 consecutive drop tests).

b) The frequency response (the Bode plot for the time response shown above).

response should produce a transform which is a straight line (see section 2.2.4). The characteristics of the responses to longer impulses can be determined theoretically (Fig.2.10), and then convolved with the transform of the drop test. These transforms can then be compared with transforms of the drop test. These resultant transforms generally have smaller resonant peaks (realised as an apparent increase in the coefficient of damping) than waveforms produced using the mathematical model. The result of deconvolution of pressure waves with these transforms would be that oscillations at the resonant frequency are not completely removed from the reconstituted waveform.

Another way of assessing the drop test is to obtain the Fn and h values for a system using the pop test and then using a mathematical model (see section 2.2.2), generate the theoretical system response characteristics, which can then be compared to those of the drop test. If one of the transforms is then subtracted from the other, the resultant transform shows the difference between the two (Fig.2.11). The same type of assessment can be obtained by comparing the differentiated Pop test response with the drop test.

Sampling of the drop test at 4000 Hz produced a drop test whose FFT resembled a more complex type of impulse response, however, downsampling to 1000Hz produced a drop test whose transform was acceptably close to an impulse response.

e) The frequency response characteristics of the catheter/transducer and the effects of using different catheters.

The resonant frequency and coefficient of damping varied with the length of the catheter, as shown in Fig.2.12, and the radius of the tubing in the way shown in table 1 at the end of this section.

For subsequent studies in the rat, a catheter was required with a resonant frequency greater than 200Hz, which would fit easily into the left ventricle without significantly altering ventricular function i.e with a diameter <1mm. A 90mm nylon catheter (Portex 800/200/175) was considered suitable.

The use of degassed saline in the catheter and transducer , significantly improved the frequency response characteristics for one catheter-transducer system e.g. the damping coefficient was reduced from 0.1 to 0.075, and the resonant frequency increased from 200Hz to 234Hz when

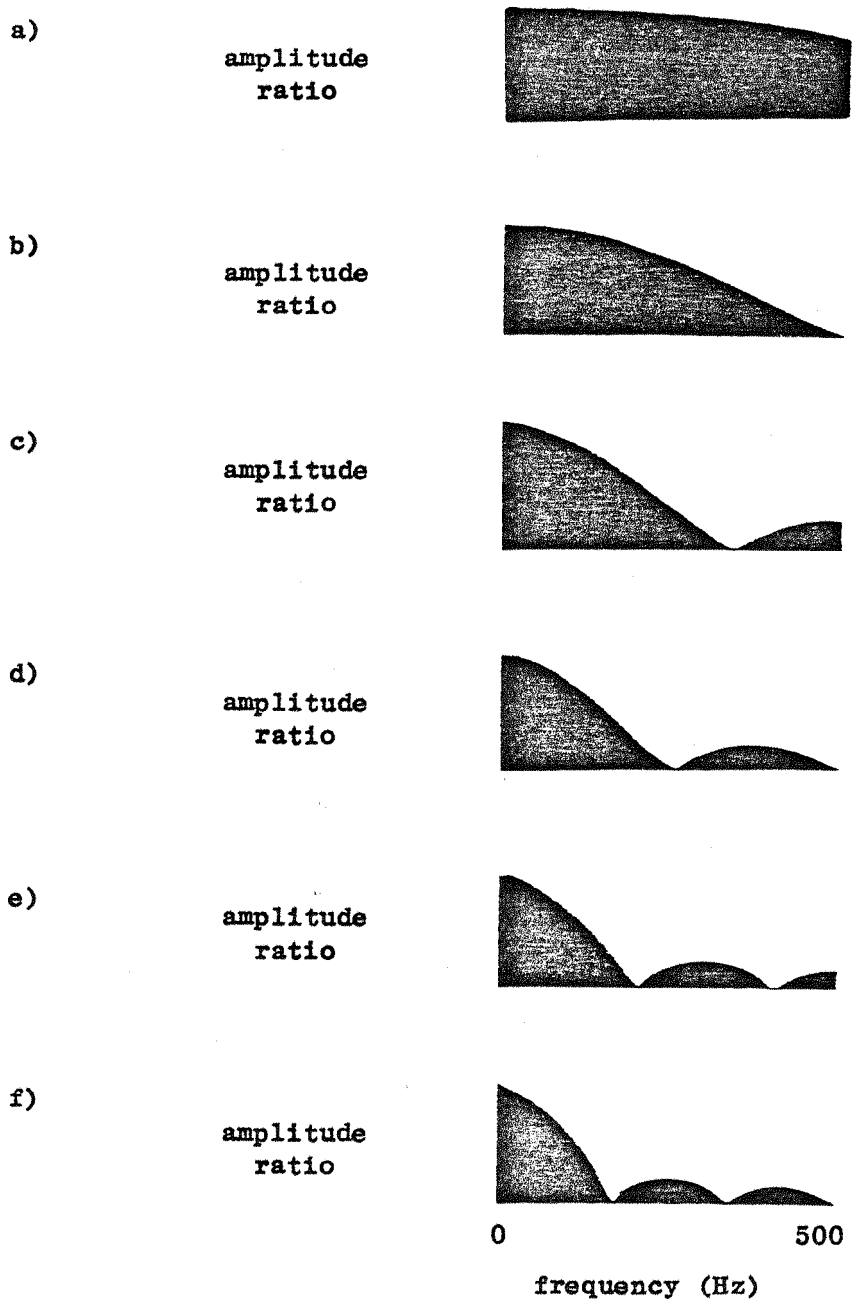


Fig. 2.10
The theoretical alterations in the amplitude vs frequency relationship of the impulse response function with increasing duration of the impulse.

a) impulse duration of 2 samples
b) " " 4 "
c) " " 6 "
d) " " 7 "
e) " " 8 "
f) " " 10 "

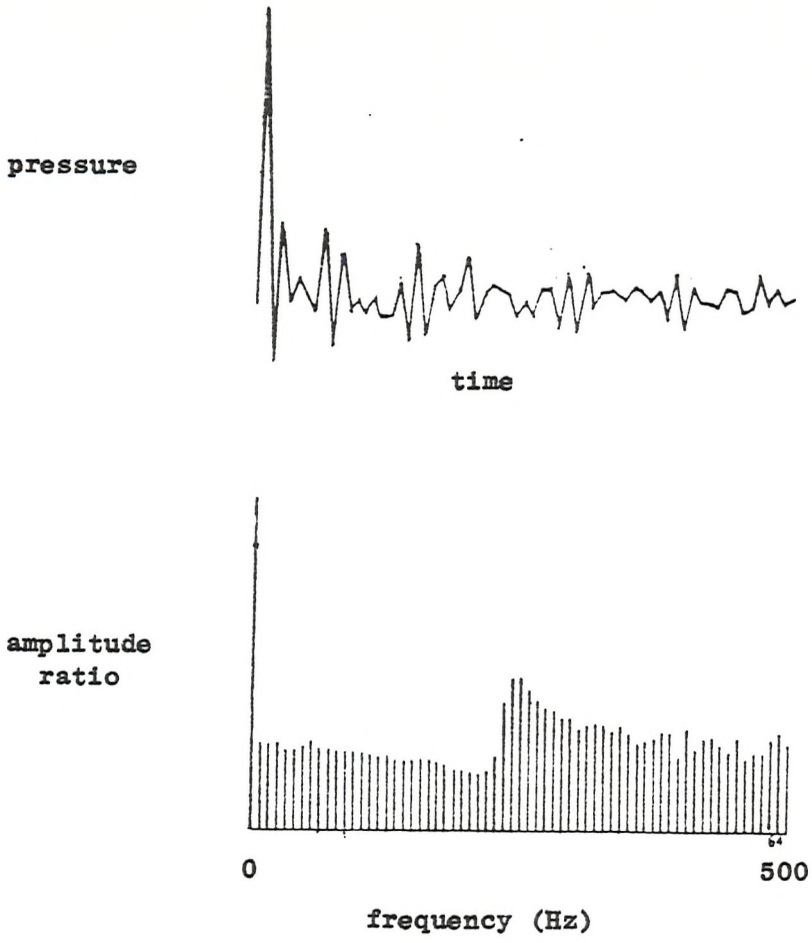


Fig. 2.11
The transform obtained by the deconvolution of the transforms of the drop test and the differentiated pop-test. In theory this amplitude vs frequency plot would be a perfectly straight line if the impulse response were ideal. However, when reconstructed in the time domain, it is clearly visible that the impulse is one sample in duration.

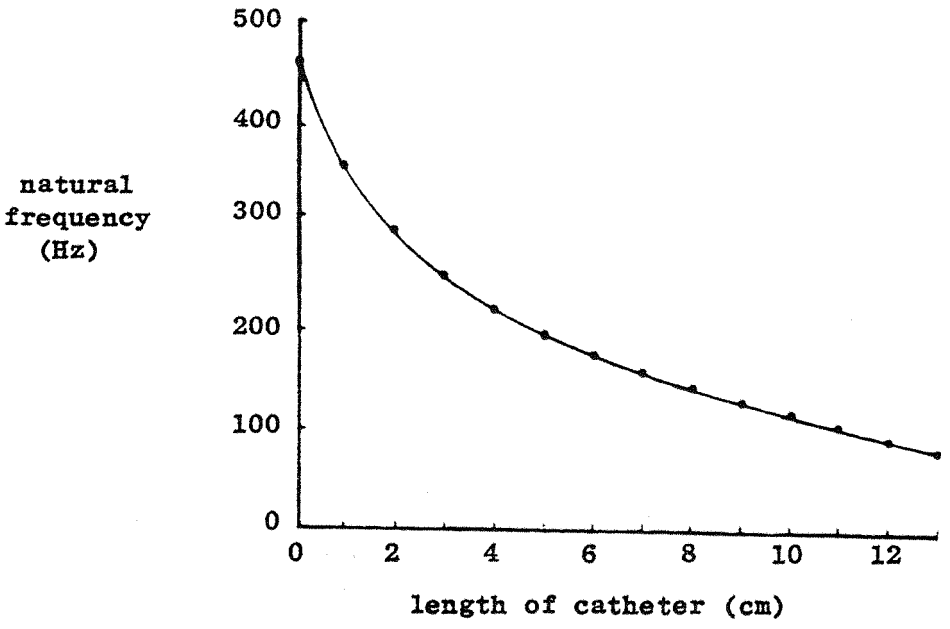


Fig. 2.12
The variation of natural frequency (fn) of the pressure measurement system with catheter length. The catheter used was Portex 800/200/200 (see table 2.1 for details).

degassed saline was used.

One of the potential problems when using a fluid-filled catheter *in vivo*, is the difficulty in preventing blood from entering the catheter and causing excess damping. This problem is potentially serious because the extra damping introduced by the blood will not be corrected for by the deconvolution of the signal. In an attempt to prevent this, 0.9% saline was slowly infused through the catheter, via the three-way tap. Unfortunately, this infusion system greatly impaired the frequency response characteristics of the system, as shown in Fig.2.13. In the light of these results the infusion was not used, and the catheter was briefly flushed before taking any pressure measurements.

Table 1 - The frequency response characteristics of various catheters

Internal diameter	External diameter	Portex Ref.	Resonant frequency	Coeff. of damping
No Catheter				
-	-	-	562 Hz	0.02
POLYTHENE cannula tubing:				
0.28mm	0.61mm	800/100/100	85 Hz	0.28
0.40mm	0.80mm	800/100/140	109 Hz	0.19
NYLON FLEX cannula tubing:				
0.62mm	1.02mm	800/200/150	187 Hz	0.09
0.75mm	0.94mm	800/200/175	234 Hz	0.07
1.00mm	1.34mm	800/200/200	273 Hz	0.06
PTFE cannula tubing:				
0.60mm	1.20mm	-	187 Hz	0.09

All of these catheters were 90mm in length and were tested with the same transducer.

2.4.2 The measurement and correction of LVP waveforms.

A typical uncorrected left ventricular pressure waveforms from the anaesthetised rat model is shown in Fig.2.14. These waveforms were recorded using an underdamped catheter/transducer system, so the characteristics of resonance and overshoot are present in these waveforms.

The comparison of LVP pressure waveforms before and after correction is shown in Fig.2.15. This shows the measured LVP waveform, with the corrected LVP waveform superimposed. The corrected left ventricular pressure waveform differed from the measured waveform in several

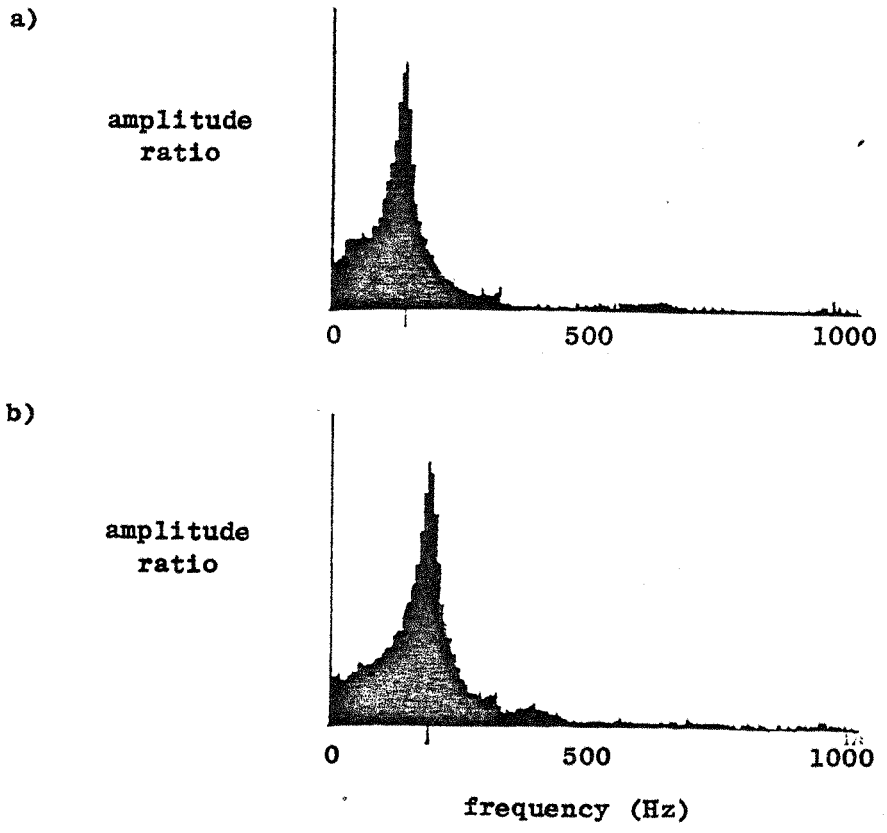


Fig. 2.13
The effect of a continuous infusion of saline through the catheter on the frequency response characteristics of the system as determined by the drop-test:

a) the amplitude vs frequency relationship of the system with the infusion switched off,
b) with the continuous infusion mechanism switched on.

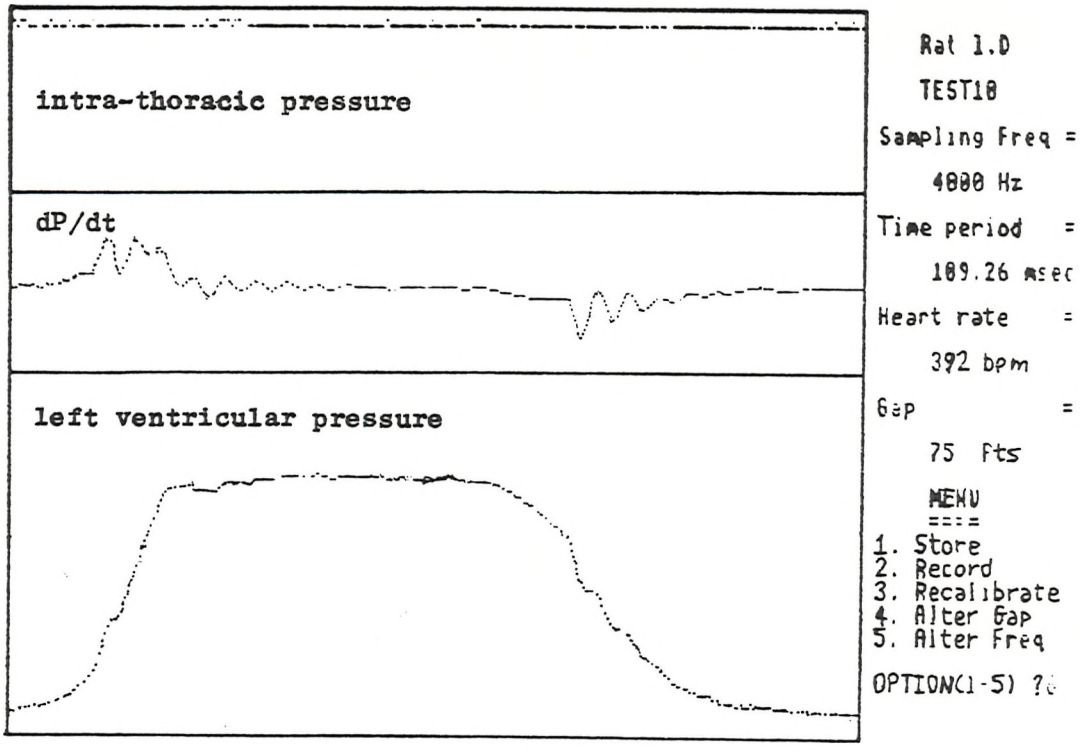


Fig. 2.14
A typical left ventricular waveform obtained from an anaesthetised rat using the pressure measurement system described in this chapter.

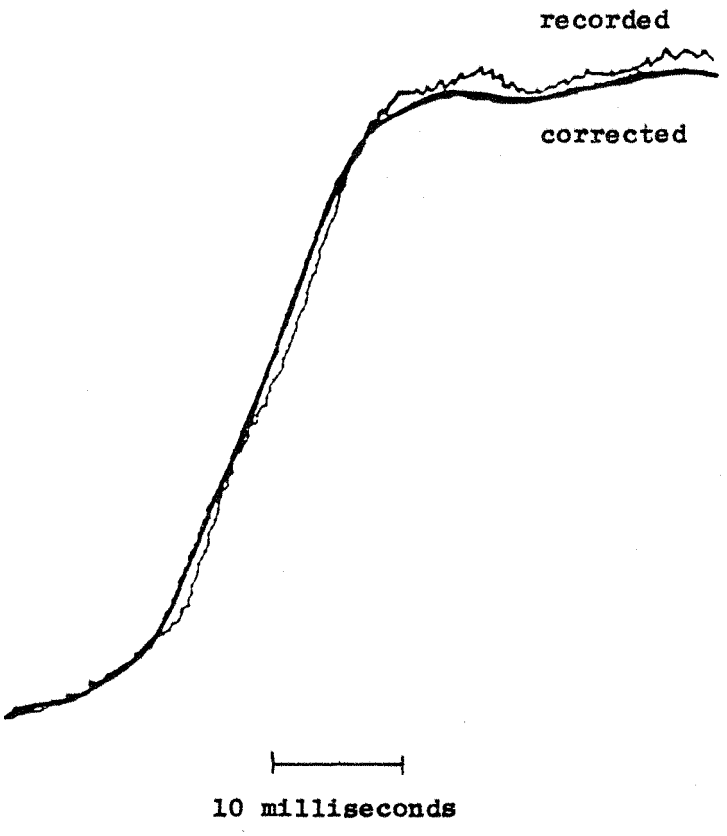


Fig. 2.15
The superimposed image of a LVP waveform before and after harmonic correction. The original measured LVP waveform shows the underdamped nature of the measuring system, as it overshoots and oscillates about the actual pressure level.

ways: elimination of the time delay shifted the corrected signal forward in time; removal of resonance lowered the peak pressure by preventing overshoot; and smoothing of the signal was accomplished by the low pass filtering due to reconstitution of selected components of the transform. Fig.2.16 shows the effect that filtering and correction have on the upsweep of a typical LVP waveform. Fig.2.16a shows the uncorrected pressure pulse. This pulse shows small oscillations caused by resonance in the recording system, and even smaller irregularities caused by noise. Reconstitution of the pressure wave by inversing the whole FFT produces the same pressure wave, but reconstitution using only the first 40 components removes the high frequency noise from the pressure wave (Fig.2.16b), effectively acting as a low pass filter with cutoff frequency at 312.5Hz. Reconstitution of the FFT after dividing by the FFT of the measuring system characteristics i.e. the drop-test, gives a pressure waveform which theoretically is free of catheter/transducer induced artifacts (Fig.2.16c). The effect of correction on the maximum LVP is shown below:-

Measured LVPmax = 103.4 $\pm$ 4.7 mmHg, (n=12)
Corrected LVPmax = 97.1 $\pm$ 4.6 mmHg, (n=12)

When analysed by paired Student 't' test this 6.2% decrease in peak LVP was found to be statistically significant ($P<.001$).

The Fourier transform of a typical corrected left ventricular pressure waveform is shown in Fig.2.17. Most of the energy of a left ventricular waveform resides in the lower frequency range. A plot of cumulative total of the energy contained in the transform against number of components of the transform used is shown in Fig.2.18. It is apparent that frequencies up to 80 Hz were necessary to ensure 95% regeneration of the pressure wave.

2.4.3 Differentiation of the LVP waveform.

a) dP/dt.

Fig.2.19 shows the waveforms obtained by differentiation of both measured and corrected left ventricular pressure waveforms. It can be seen that the uncorrected waveform gives an over-estimate of the peak left ventricular dP/dt. Average values of these measurements were:

dP/dt_{max} (measured LVP) = 8145 $\pm$ 538 mmHg/sec (n=12)
dP/dt_{max} (corrected LVP) = 7795 $\pm$ 530 mmHg/sec (n=12)

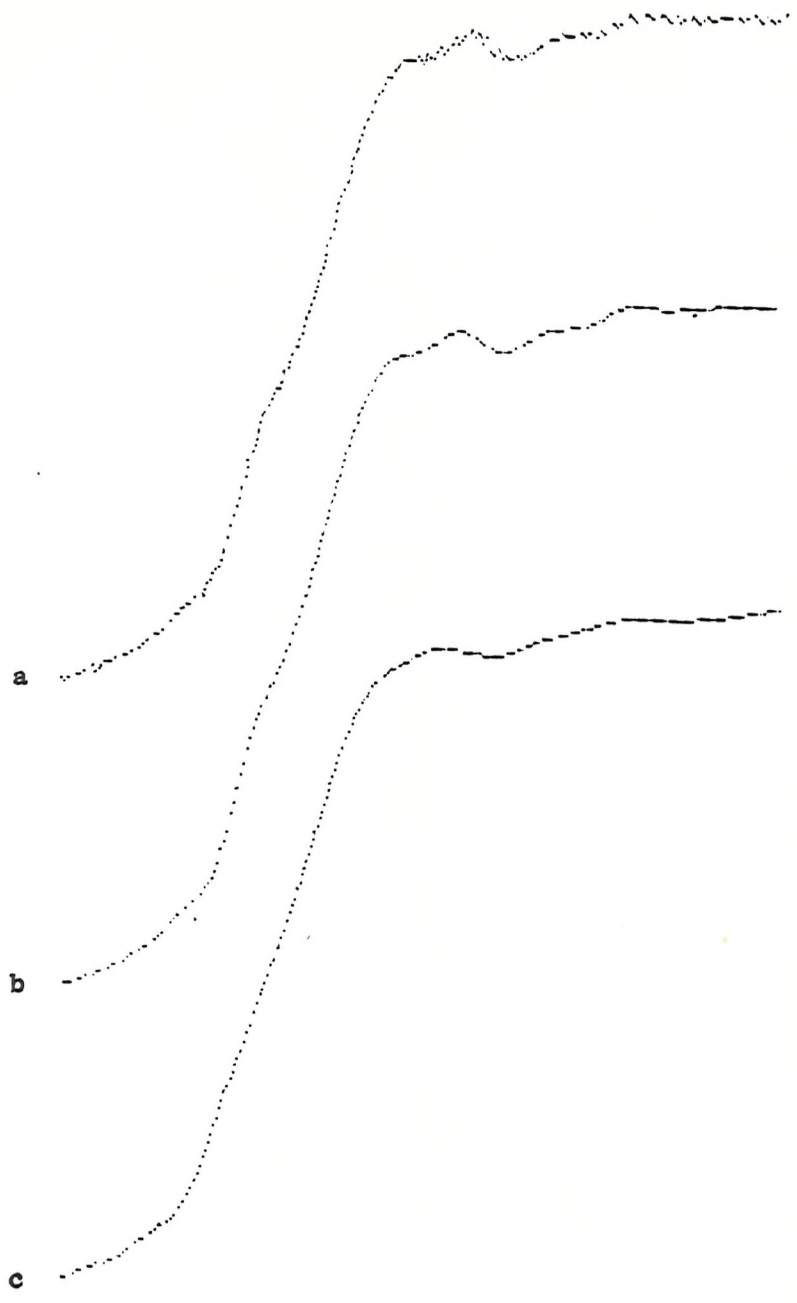


Fig. 2.16
The effect of filtering and deconvolution on the upsweep portion of a typical LVP waveform.

a) the original measured waveform
b) the filtered waveform (>250Hz)
c) the waveform after harmonic correction.

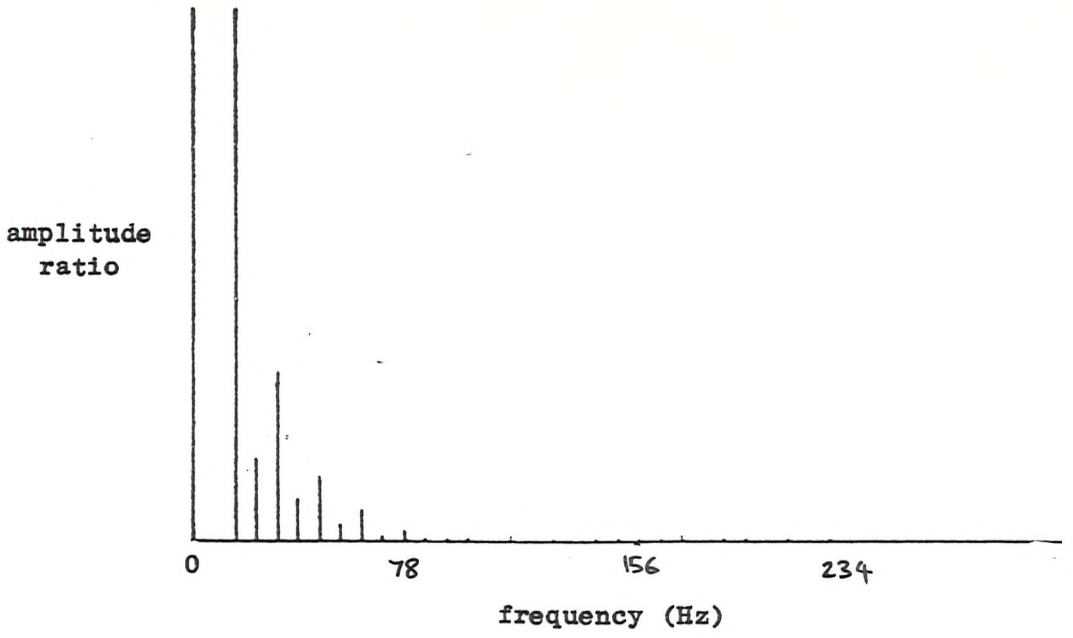


Fig. 2.17
The frequency content of a measured LVP waveform.

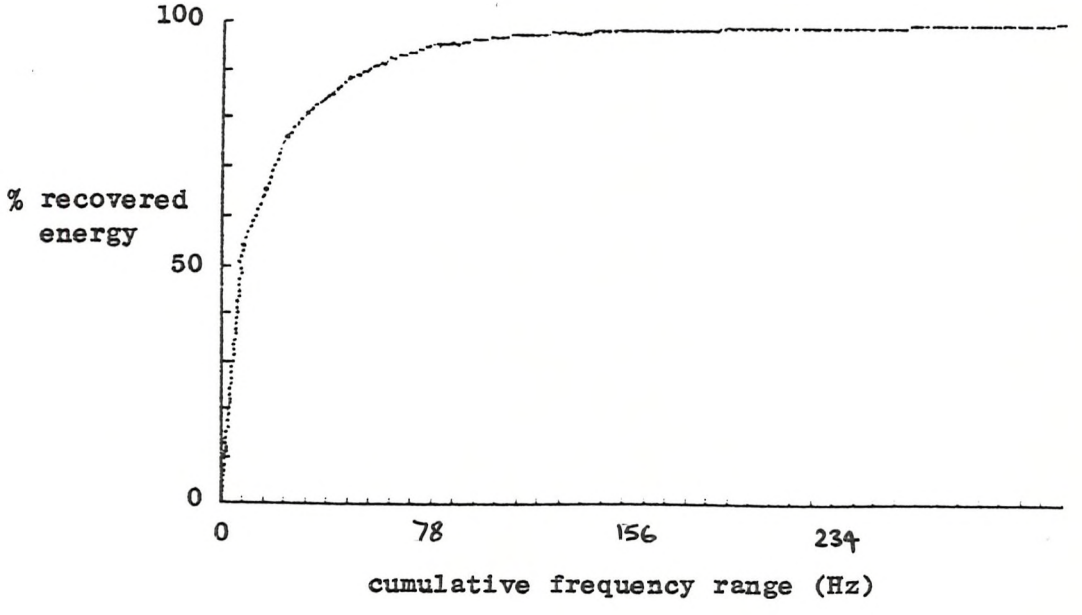


Fig. 2.18
Cumulative total energy in a LVP waveform.

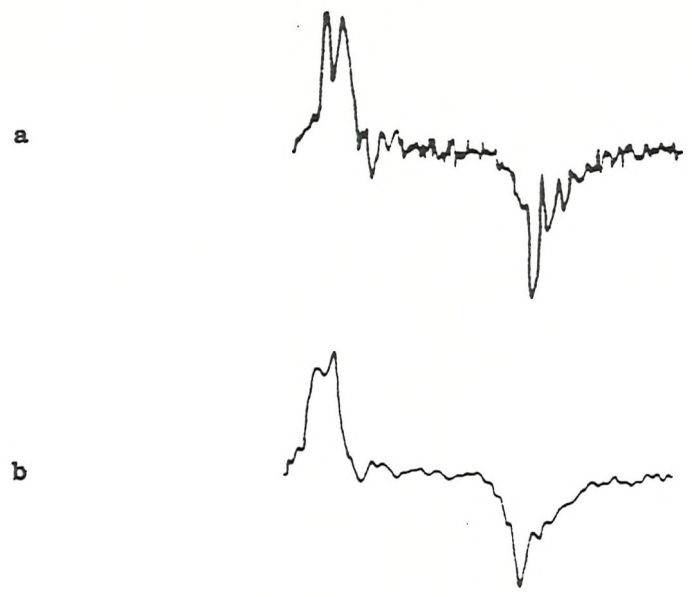


Fig. 2.19
The first differentials of:
a) a measured waveform,
b) a corrected waveform.

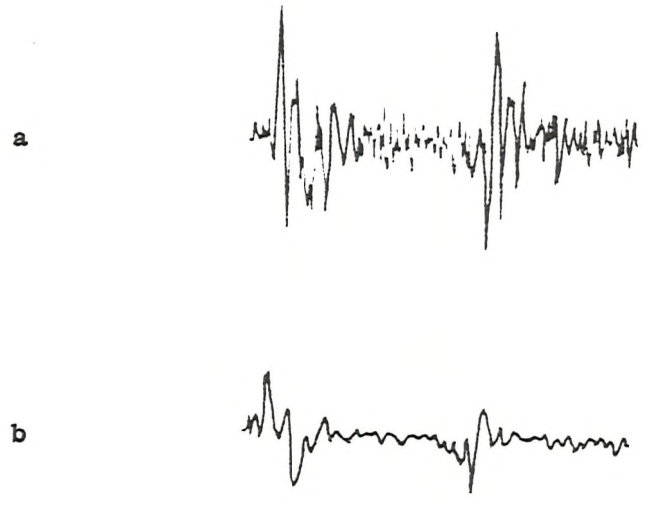


Fig. 2.21
The second differentials of:
a) a measured waveform,
b) a corrected waveform.
waveforms.

When analysed by paired Students 't' test, this 4.4% decrease in dP/dtmax was found to be statistically significant (P<.001).

The variation of the peak value of dP/dt with the number of components used for reconstitution is shown in Fig.2.20.

b) d^2P/dt^2

Fig.2.21 shows the waveforms obtained by differentiation of the measured and corrected dP/dt waveforms. It can be seen that the uncorrected waveform is much noisier and overestimates the peak left ventricular d^2P/dt^2. Average values of these measurements were:

d^2P/dt^2_{max} (measured LVP) = 999 + 83 $mmHg/sec^{-2}(x10^3)$, n=12

d^2P/dt^2_{max} (corrected LVP)= 940 + 84 $mmHg/sec^{-2}(x10^3)$, n=12

When analysed by paired Student 't' test this 6.1% decrease in d^2P/dt^2 was found to be statistically significant (P<.001).

The variation of the peak value of d^2P/dt^2 with the number of components used for reconstitution is shown in Fig.2.22.

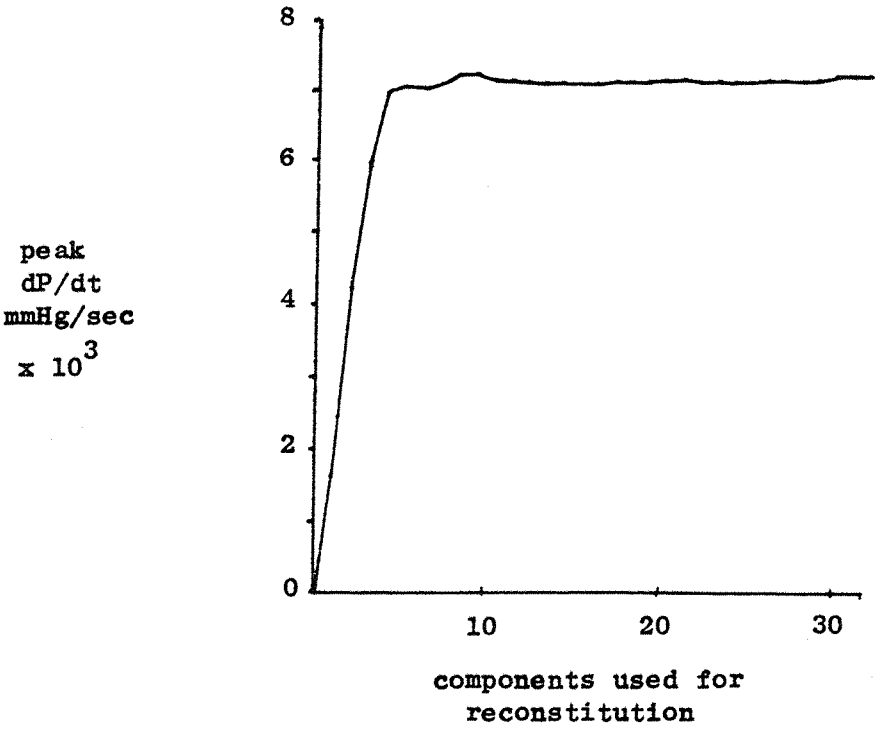


Fig. 2.20
Variation of peak dp/dt with components used for reconstitution

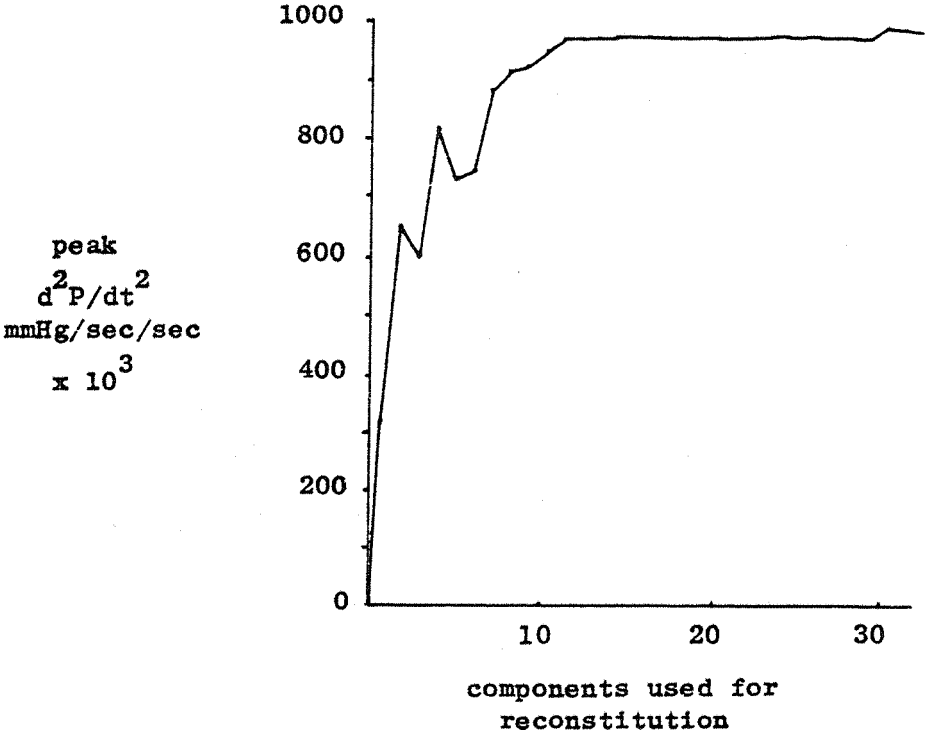


Fig. 2.22
The variation of peak d^2P/dt^2 with components used for reconstitution.

2.5 DISCUSSION.

The most commonly used techniques for the determination of the frequency-response characteristics of catheter/transducer systems are the balloon pop-test (Wood, 1950 ; Shapiro & Krovetz, 1970) and oscillation chamber (Noble, 1959; Stegall, 1967). The pop-test allows the determination of the resonant frequency and damping coefficient, but the response to a pop-test could not be used directly in the deconvolution of recorded signals because a balloon burst gives a pressure step not an impulse response, and the exact time of the burst could not be defined. Oscillation chambers apply vibrations to the catheter tip at a selected frequency while the system response is recorded; this is repeated for different frequencies, allowing the compilation of graphs similar to Fig.2.9; unfortunately this method is very laborious and requires specialised equipment that was not available.

The drop-test, however, is much simpler to apply than the pop-test or oscillation chamber, and conveniently allows the frequency-response characteristics of the measuring system to be estimated. It also gives a response that can be used, unmodified, in the deconvolution of experimental pressure recordings. An advantage of this procedure is that the catheter / transducer may not behave exactly like a simple damped oscillator but because the response to a drop-test does not rely upon such a model, it more completely describes the system characteristics. The process of deconvolution should therefore largely remove those characteristics, irrespective of their nature.

Van Den Bos, Elzinga, Westerhof, and Noble, (1973) stated that the analysis of single heartbeats should be avoided because of possible variation between consecutive beats. In our experience, however, inter-beat variation was related to fluctuations in intra-thoracic pressure. To eliminate this source of variation, heartbeats were recorded only during periods of stable ITP (i.e. towards the end of passive expiration).

Wiggers'(1927) proposal that left ventricular dP/dt_{max} is an index of myocardial contractility, has stimulated considerable research. Lambert, Nichols, and Pepine (1983) compared many different indices of contractility, and ranked them according to their sensitivity to an inotropic agent and insensitivity to changes in preload and afterload: dP/dt_{max} and d^2P/dt^2_{max} were rated 10th and 4th respectively,of 24 indices. These

two indices are simple to derive from the left ventricular pressure wave and were therefore used in the present study.

Few reports of left ventricular function in the rat have appeared in the literature (Pfeffer, Pfeffer, Fishbein, Fletcher, Spadaro, Kloner, and Braunwald, 1979; Zimmer, 1984; Van der Werf, Noakes and Douglas, 1985). Zimmer found left ventricular dP/dt_{max} to be 6073 +/- 187 mmHg/sec in the Inactin anaesthetised rat, using a catheter-tip manometer, which is likely to give very accurate measurements. This is similar to the value reported in the present study. However, using a conventional fluid-filled catheter pressure transducer, Zimmer found dP/dt_{max} "to be around 13,000 mmHg/sec" and Pfeffer et al similarly found dP/dt_{max} to be around 13,500 mmHg/sec. These values are much higher than those reported in the present study and those obtained with catheter tipped manometers. This is consistent with the findings reported in the present paper, that resonance in the catheter/transducer system may lead to overestimation of dP/dt_{max}. Van der Werf et al, (1985), measured ventricular dP/dt in the isolated perfused rat heart and found it to vary between 6000 and 8000 mmHg/sec. These workers reported that their earlier studies had yielded values between 3000 and 4000 mmHg/sec, but they discovered that their values of dP/dt_{max} were being limited by the poor frequency response characteristics of their pressure measuring system. These studies emphasise that variation in the frequency response characteristics of measuring systems can cause inconsistances between reported values for dP/dt in the rat.

Left ventricular dP/dt_{max} has been more widely reported in larger species, such as guinea pig (Scott Hayes, 1982), rabbit (McRitchie, Blood, and Chalmers, 1984), dog (Wallace, Skinner, and Mitchell, 1963) and man (Gleason & Braunwald, 1962; Mason, 1969). The basal heart rate is considerably faster in the rat than any of these species , yet the peak systolic pressure is comparable. It is inevitable, therefore, that the dP/dt_{max} in the rat will be greatest.

The present study shows that the resonance and damping characteristics of the catheter/transducer system can be measured by the drop test and the technique of deconvolution can be successfully employed to minimise or eliminate the errors due to these characteristics which arise whilst recording LVP from the rat with a standard catheter/transducer system. Ten components of the FFT (i.e. sine waves up to 78.1 Hz) were necessary to give

reliable reconstitution of the left ventricular pressure wave, but 33 components (i.e. sine waves up to 260 Hz) were necessary if the reconstituted pressure waves were to yield reliable estimates of dP/dt.

CHAPTER 3:
The *in vivo* measurement of indices of myocardial contractility.

3.1 GENERAL INTRODUCTION.

The previous chapter described the development of a system for taking high fidelity measurements from the rat heart *in vivo*. This chapter describes experiments in which the left ventricular pressure data were used to calculate various indices of left ventricular contractility, since there was little information available in the literature concerning the basal levels of these indices in the anaesthetised rat.

Ideally an index of contractility should be sensitive to factors known (or believed) to alter cardiac contractility, but insensitive to factors which change other aspects of circulatory function. Unfortunately most, if not all, indices are influenced by factors other than the contractility, so I next investigated the sensitivity of these indices to changes in the contractile state of the myocardium of the rat. Experiments were performed to investigate the relative dependence of the indices on changes in the preload and afterload on the heart, since these are factors which may change during the early post-burn period.

These experiments also provided an opportunity to compare the relative merits of the various indices of contractility.

3.2 LITERATURE REVIEW.

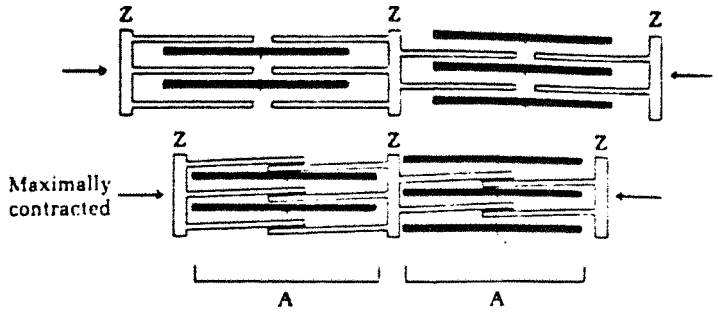
3.2.1 The biochemical basis of muscle contraction.

The basic contractile unit within cardiac muscle has been well characterised. Each unit consists of the structural proteins actin and myosin (which form the thin and thick filaments respectively), and the regulatory proteins troponin and tropomyosin. These proteins are arranged in a regular, repeating manner to form sarcomeres (Fig.3.1). Force generation and sarcomere shortening occur by the sliding filament mechanism (Huxley, 1974). The formation of cross bridges between the actin and myosin filaments is initiated by calcium ions. The calcium ions bind to sites on the troponin molecule leading to a change in the adjacent tropomyosin molecule, thus revealing the active sites on the actin filament and allowing myosin to bind and cross bridges to be formed (Hitchcock, Huxley & Szent-Gyorgi, 1967). The binding of myosin to actin leads to ATPase activity, the generation of force and the shortening of the sarcomere. The detailed mechanisms of cross bridge cycling and sarcomere mechanics are reviewed elsewhere (Lymn & Taylor, 1971). Changes in force of contraction depend on the number of cross-bridges that can form, which, in turn, depends on the overlap of filaments, the intracellular availability of calcium ions and the binding of calcium ions to troponin.

Regular contractions of the heart are initiated by cardiac action potentials. The action potentials are due to a fast inward current caused by sodium entry and then a second slow inward current (I_{si}), caused mainly by the influx of calcium ions through sarcolemmal, voltage dependent calcium channels. This influx of calcium does play a role in the contractile process, although a complex one, but the amount which enters during an action potential could cause only 10-15% of the observed maximum generated force (Winegrad, 1982). The main source of sarcoplasmic calcium is the sarcoplasmic reticulum (SR). Release of calcium from the SR may be activated by calcium-induced calcium release (Fabiato & Fabiato, 1978).

Elevation of the sarcoplasmic calcium concentration is the trigger for cardiac systole and the removal of calcium ions from the sarcoplasm is the cause of the subsequent diastole. The main mechanism of calcium removal is via calcium dependent ATPase's which pump calcium ions back into the SR. Some calcium is also removed via extrusion into the ECF by sarcolemmal calcium ATPase's and

a)



b)

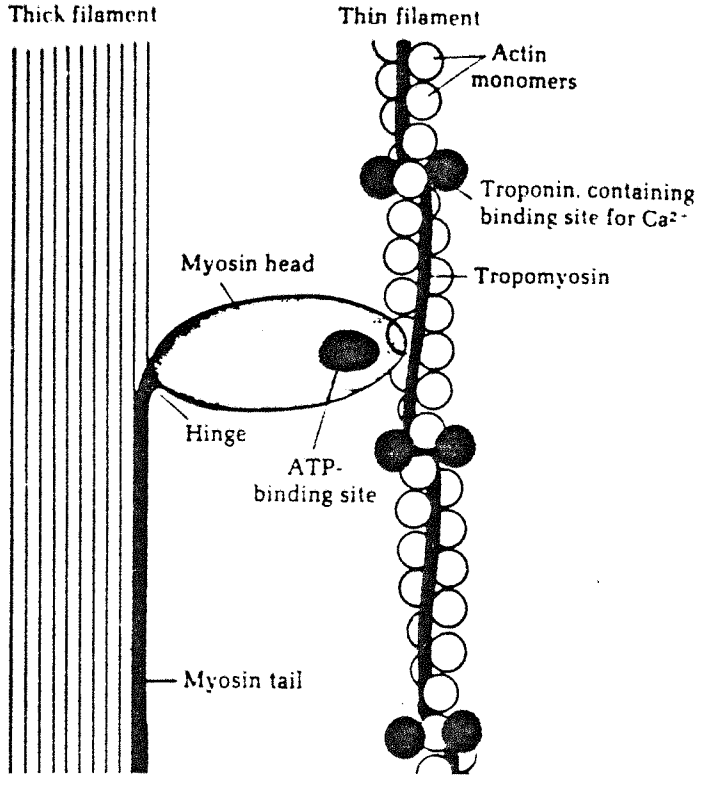


Fig. 3.1
The structure of muscle:

a) the ultrastructure of the sarcomere,
b) the interaction of the contractile proteins.

a sodium-calcium exchanger. These mechanisms keep the resting calcium concentration at approximately 100nM, which is low enough to ensure complete relaxation during diastole.

The contractility (ability to develop force) of the heart can be altered by the autonomic nervous system. The ventricle receives a dense sympathetic innervation which releases noradrenaline that acts at the cellular level on B1-adrencreceptors. Sympathetic or adrenergic stimulation of the heart will cause an increase in contractility. This is characterised in isolated heart muscle preparations as an increase in the maximum force generated (F_{max}), the rate of generation of force (dF/dt_{max}) and the rate of relaxation ($-dF/dt_{max}$). At the cellular level, β adrenoreceptor stimulation causes an increase in the intracellular concentration of cAMP. This acts as a secondary messenger which activates specific protein kinases and leads to :-

i) An increase in I_{si} through increased recruitment of voltage dependent sarcolemmal calcium channels.

ii) An increase in the rate of removal of sarcoplasmic calcium via potentiation of calcium exchange mechanisms.

iii) A decrease in the duration of contraction brought about by alterations in the calcium-troponin binding characteristics.

iv) Alterations in the kinetics of cross-bridge cycling brought about by changes in the sensitivity of the myosin ATPase.

v) An increase in metabolism which couples energy availability to metabolic requirements.

The regulation of contractile performance through protein phosphorylation (as summarised above) has been reviewed by Demeille & Pechere (1982).

There is also evidence of a cholinergic innervation to the ventricles. Activation of muscarinic receptors could cause a physiological antagonism of the B-adrenergic system via a c-GMP mediated mechanism (Watanabe, 1985).

3.2.2 Indices of contractility.

Myocardial contractility is a term which has existed since the turn of the century to describe the force developed by the contracting ventricle. A definition of contractility which is commonly used today is "the intrinsic ability of the heart to function, other than that induced by changes in pre-systolic fibre length i.e factors other than the Frank-Starling relationship" (Rothe, 1976). An indicator of ventricular performance has

long been sought which is insensitive to changes in end-diastolic fibre length (preload), whilst being sensitive to alterations in the contractile state, whether produced by intrinsic or extrinsic mechanisms. Such an ideal index of myocardial contractility has yet to be discovered for in vivo preparations. In fact, evidence has emerged which suggests that such an index could not possibly exist since contractility and the Frank-Starling mechanism are inextricably linked; experiments using skinned cardiac muscle fibres have suggested that the Frank-Starling mechanism cannot be explained solely by the overlap of thin filaments, but is at least partly due to length dependent activation of the contractile apparatus (Fabiato & Fabiato, 1975). This conclusion is further substantiated by the finding of Ter Keurs, (1977) that the level of activation caused by calcium ions is greater at longer sarcomere lengths than short ones. It may well be more realistic therefore to search for an index which varies in a predictable manner with preload (Abel & McCutcheon, 1979).

The concept of differing contractile states was very clearly defined by Sarnoff & Berglund (1954), who constructed left ventricular function curves in which stroke work (stroke volume vs aortic pressure) was plotted against end-diastolic pressure. This produced a curve which demonstrated the Frank-Starling relationship, i.e. the ability of the myocardium to change its work output in response to changes in preload and afterload. Changes in the myocardial contractility caused by sympathetic stimulation were apparent as displacements of these curves (see Fig.3.2), each curve defining a different contractile state. Ventricular function curves, however, suffer from problems inherent in the methodology employed to construct them. The contractility of the myocardium and the arterial pressure must be held constant during large changes in preload in order to produce the data for the function curves and this is very difficult to achieve.

The left ventricular pressure is relatively simple to measure so many indices of contractility have been devised which are derived from such pressure measurements. The following indices were used in the experiments described in this and the next chapter.

a) dP/dt_{max}.

One of the earliest indices of contractile state to be suggested was the maximum rate of development of left ventricular pressure, dP/dt_{max} (Wiggers, 1927). It was observed that an increase in inotropic state caused an increase in the rate of development of tension (dT/dt)

in ventricular muscle. This observation was extended to apply to the intact heart as follows. It is evident from the law of Laplace that during an isovolumic contraction, dP/dt will be related to the rate of development of tension (dF/dt) in the muscle and also to the dimensions (i.e. diameter) of the ventricle (D), as shown in Eqn (1)

(1) $$dP/dt = (dF/dt) / D$$

This implies that dP/dt should decrease as the length of the muscle fibres increases if dF/dt were constant i.e. if there were no change in contractility. But of course the Frank-Starling relationship produces an increased dF/dt with increasing fibre length, which would tend to oppose the former. If these two balance, dP/dt will be independent of the ventricular dimensions and hence preload. If dP/dt_{max} occurs during isovolumic contraction i.e before the opening of the pulmonary or aortic valves, it will be independent of afterload (Van Den Bos et al, 1972). Thus in principle, dP/dt_{max} should provide a good index of contractility. In practice, however, dP/dt_{max} is observed to vary with preload (Mason, 1969; Nejad et al, 1971). Another disadvantage when using this index is that aortic valve opening and peak dP/dt are often very close together; when the aortic pressure decreases the aortic valve opens before the time that the heart is capable of its maximum dP/dt so the peak value of dP/dt will apparently fall. There is also the problem in intact unpaced preparations that heart rate may influence the contractility since Wallace et al, (1963) have shown that in areflexic canine preparations in which the atrium was paced, there was a direct linear correlation between heart rate and dP/dt_{max}.

b) d^2P/dt^2_{max}.

The peak value of the second derivative of left ventricular pressure is reported to be a sensitive indicator of inotropic state (Letac et al, 1968; Lambert et al, 1983). The main peak of the d^2P/dt^2 signal generally occurs between one fifth and one sixth of the time between the start of systole and the maximum left ventricular pressure and corresponds to the point of maximum acceleration attained by the left ventricular pressure during isovolumic contraction. Because the first and second derivatives are obtained from different portions of the left ventricular pressure waveform and these indices differ in their dependence on preload and

afterload, it has been suggested that they may have quite different significance for the contractile process (Letac et al,1968). The peak value of $d^2p/dt^2/p$ has also been proposed as an index of contractility and has been reported to be independent of preload and afterload over a wide range of end diastolic pressures in the canine heart-lung preparation (Nejad et al, 1971).

A major problem with the measurement of d^2P/dt^2 is the noise which is generated by differentiation. The second differential is usually very noisy and filtering is essential to obtain a smooth trace, but filtering considerably reduces the sensitivity of the index. Letac et al, (1968) measured d^2P/dt^2 using a fibreoptic catheter (with good frequency response characteristics at frequencies up to 3000Hz) together with an electronic differentiating circuit. They found that a 600Hz low pass filter decreased the amplitude of the signal by 40%, yet it is doubtful if most pressure measuring systems with conventional transducers would be reliable at frequencies much above 300Hz.

c) V_{max}.

The force developed in a contracting muscle fibre is dependent upon the number of cross bridges interacting with the thin filaments at any one time, so measurements that depend on the velocity of shortening could provide an index of contractility. To be cautious, it is worth noting that the velocity of contraction is also determined by the kinetics of cross bridge association and dissociation; the maximum velocity of shortening also depends on the maximum turnover rate of the individual cross bridges. So it follows that increasing the number of cross bridges might not necessarily increase the maximum velocity of contraction. However, in isolated papillary muscle, V_{max} correlates well with the rate of ATP hydrolysis by myosin (Winegrad, 1979), so these potential problems may not be important.

Many investigations into the maximum velocity of contraction (V_{max}) have been based on the Hill model for contraction of skeletal muscle (Hill, 1939). This assumes that each circumferential muscle fibre (CF) consists of a contractile element (CE) and a series elastic element (SE). This model predicts the relationship in Eqn (2):

(2) $$V_{CE} = V_{CF} + V_{SE}$$

where :
V_{CE} = Velocity of contractile element shortening.
V_{CF} = Velocity of circumferential fibre shortening.
V_{SE} = Rate of lengthening of the series elastic element.

and by further rearrangement Eqn(3)

(3) $$V_{CE} = (dP/dt) / k.P$$

where: k = Coefficient of stiffness.

This model produces three possible indices of contractility, V_{CE}, V_{PM} (which is peak V_{CE}) and V_{max} (which is obtained by the extrapolation of V_{CE} to zero pressure generation on a plot of V_{CE} *vs* LVP).

In order to accomodate the fact that cardiac muscle can have a resting tension during diastole, a new model was introduced with a third element, the parallel elastic element (PE) which resides in parallel with the other two elements (CE & SE). This model was named the 'Maxwell' model and the new element represents the property of cardiac muscle to oppose a force stretching the muscle. Mathematically this alteration of the model causes the substitution of developed pressure (developed pressure = absolute pressure - LVEDP) for absolute pressure. Typical plots of V_{CE} *vs* LVP for absolute and developed pressure are shown in Fig.3.3.

These indices have been analysed and compared in many studies (Pollack,1970; Mason *et al*, 1971; Peterson *et al*, 1973; Urschel *et al*, 1980). There is great debate concerning the usefulness of V_{max} and its related indices *in vivo*. Initial studies indicated that Vmax remained unchanged during alterations in preload and afterload whilst inotropic substances did change the index (Fry *et al*, 1964). The underlying assumptions required to extrapolate from isolated muscle to intact heart have been criticised, however, especially on theoretical grounds. The coefficient of stiffness must be linearly related to muscle stress for this index to be accurate and this may not in fact be the case. Unlike skeletal muscle , cardiac muscle does not develop force at zero load, so the value of V_{max} must be extrapolated to the pressure axis. This procedure may introduce a large error into the measurement, and investigators do not agree on the optimal method of extrapolation (Peterson *et al*, 1973; Van Den Bos *et al* 1973; Urschel *et al*, 1980).

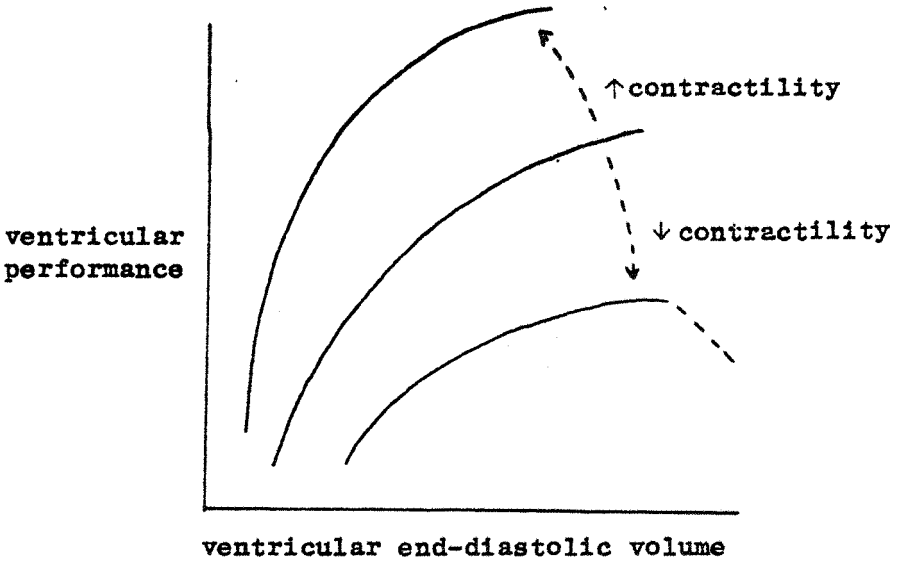


Fig. 3.2
Changes in myocardial contractility due to sympathetic nerve stimulation (from Sarnoff & Berglund, 1954).

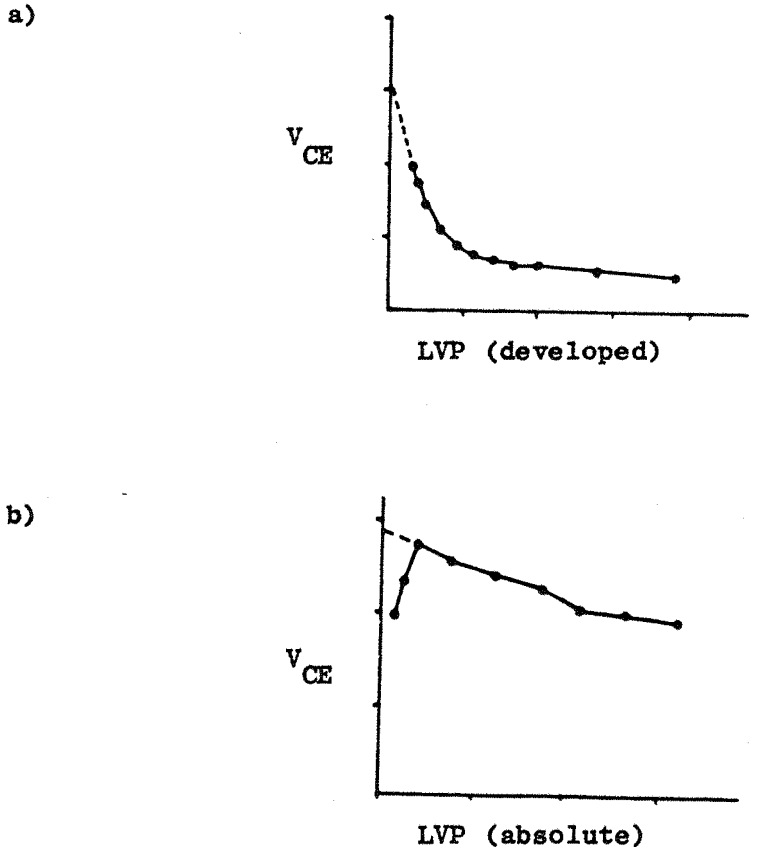


Fig. 3.3
The evaluation of V_{max} values from:
a) V_{CE} vs LVP (developed pressure).
b) V_{CE} vs LVP (absolute pressure).

d) Indices based on thermodynamic principles.

From the laws of thermodynamics, Bloomfield et al,(1975) have derived power density functions relating the free energy in the myocardium to the pressure on the blood in the ventricle. These functions represent the ability of the ventricle to do work i.e. the build up of potential energy as muscle fibres contract and stretch the series elastic element, and involve the evaluation of the work the ventricle could do at every point along the rising phase of isovolumic contraction. Since no external work is being done, the change in free energy (potential for work) is independent of load. The development of this model has produced three indices of contractility:

i) The Energy Averaged Power Density (APD).
This function is calculated as:

4)
$$APD = \frac{1}{Pmax} \int_{0}^{Pmax} P' \cdot dP$$

Where: P = Left ventricular pressure.
P'= Rate of change of LV pressure (dP/dt).

ii) The Energy Averaged Rate of Power Density Generation (ARPD).

This function is calculated as:

5)
$$ARPD = \frac{1}{P'_{max}} \int_{0}^{P'_{max}} P'' \cdot dP'$$

Where:
P' = dP/dt
P''= Rate of acceleration of LVP (d^2P/dt^2).

iii) The Frequency Normalised, Power Averaged Rate of Power Density Generation (FARPD).
This function is calculated as:

6)
$$FARPD = \frac{T^2}{P'_{max}} \int_{t(P'=0)}^{t(P'_{max})} (P'')^2 \cdot dt$$

Where: P' = dP/dt (mmHg/sec)
P''= d^2P/dt^2 (mmHg/sec^2)
T = Period of the cardiac cycle (sec/cycle)
$t(P'_{max})$ = Time at which P' is maximal.

t(p'=0) = Time at which P' is zero.

APD and ARPD were developed for use in vitro, where heart rate was controlled by pacing. Moreno et al (1979) observed that there was a direct relationship between ARPD and heart rate and they concluded that this relationship was independent of any inotropic effects or Bowditch-like phenomena) that the heart rate may have on cardiac muscle, hence the index FARPD was derived, which should minimise errors caused by changes in heart rate. APD and ARPD have been evaluated for sensitivity to inotropic stimuli and to changes in preload and afterload (Moreno et al, 1976; Lambert et al, 1983). ARPD was found to be a sensitive index in these studies and showed low sensitivity to both preload and afterload.

e) Indices of myocardial relaxation.

The peak rate of pressure decline ($-dP/dt_{max}$) and the peak deceleration of pressure ($-d^2P/dt^2_{max}$) have been measured as indicators of the state of myocardial relaxation. The determinants of ventricular relaxation were recently reviewed by Brutsaert, Housmans and Goethals (1980). These workers proposed a dual mechanism for the control of ventricular relaxation in mammalian heart. They proposed that the relaxation of the heart is governed by the continuous interplay of the sensitivity of the contractile system to the prevailing loading (load dependence) and the decaying activation (inactivation dependence).

f) Pressure-Volume relationships.

Another group of indices requires information concerning ventricular volume as well as pressure and recently great interest has been shown in the ventricular pressure volume relationship. Fig.3.4 shows that this relationship takes the form of a loop. The values of left ventricular pressure and volume at the end of systole occur at a time when the ventricular and aortic blood pressures balance and the ventricular volume is almost constant, i.e. isometric contraction. If this point of the curve is read for the different loops that occur with different preloads, then a straight line is obtained, which can be expressed mathematically as:

7) $$P_{ES} = E_{ES} \cdot (V_{ES} - V_d)$$

where: P_{ES} = Left ventricular end systolic pressure.
V_{ES} = Left ventricular end systolic volume.
E_{ES} = The Slope of the P_{ES} vs V_{ES} line.
V_d = The Ventricular volume at zero pressure.
(i.e the intercept on the volume axis)

A graph of P_{ES} vs E_{ES} is built up of values taken at different preloads, so clearly, the value of E_{ES} should be insensitive to preload. The equation also implies that E_{ES} should be insensitive to aortic impedance. However, changes in the contractile state do alter the value of E_{ES} whilst the value of V_d remains relatively constant (Sagawa, 1978). This relationship has been used to investigate changes in contractile state caused by a variety of physiological mechanisms (Suga & Sagawa,1976). The main disadvantage of this technique is that it requires the measurement of both ventricular pressure and volume, and for this reason was not used in the present study.

g) Other indices.

Many other indices of contractility have been developed including systolic time intervals (Garrard et al, 1970), isovolumetric time-tension relationships (Siegal & Sonnenblick, 1963) and indices based on the rate of change of power within the ventricle (Stein et al, 1976). Many of these different indices of contractility have been reviewed by Van Den Bos et al, (1973); Quinones et al, (1976); Abel, (1978) and more recently by Lambert et al,(1983). The latter report assessed 32 indices of contractility for sensitivity to inotropic stimuli and insensitivity to preload and afterload in the intact, paced, canine heart preparation. They reported ARPD to be the best index of contractility, d^2P/dt^2_{max} was rated 4th, APD was rated 7th and dP/dt_{max} 10th.

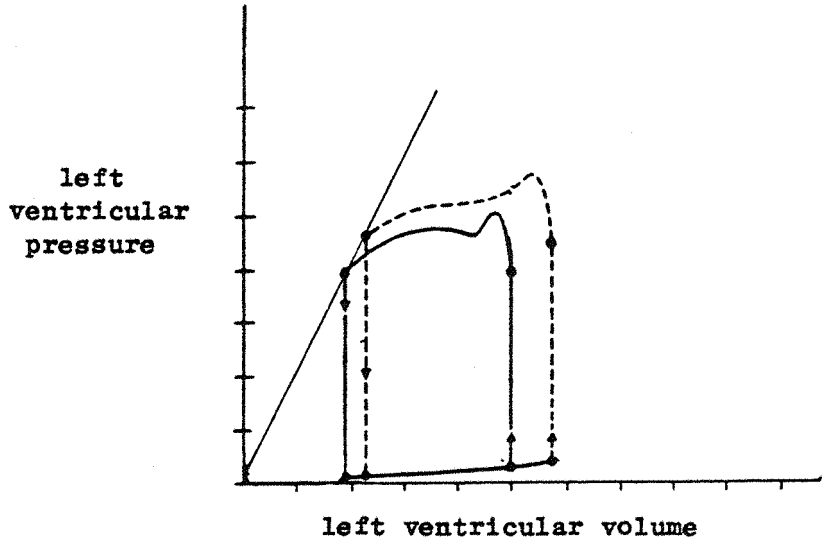


Fig. 3.4
The evaluation of E_{max} values from left ventricular, pressure vs volume relationships (from Suga & Sagawa, 1978).

3.3 METHODS.

3.3.1 The measurement and evaluation of contractile indices.

The following indices of contractility were measured: dP/dt_{max}, $-dP/dt_{max}$, d^2P/dt^2_{max}, $-d^2P/dt^2_{max}$, V_{max}, APD, ARPD and FARPD. These indices were evaluated by microcomputer from digitised recordings of left ventricular pressure waveforms, obtained using the methods described in chapter 2.

a) dP/dt_{max} and $-dP/dt_{max}$.

These were computed as the most positive and negative values of the dP/dt waveform, which was derived by the 11 point polynomial regression method of differentiation described in section 2.3.4

b) d^2P/dt^2_{max} and $-d^2P/dt^2_{max}$.

The dP/dt wave was differentiated again by the 11 point polynomial regression method as described in section 2.3.4. The d^2P/dt^2_{max} was computed as the peak value occurring in the first half of the d^2P/dt^2 waveform and the $-d^2P/dt^2_{max}$ as the most negative value occurring in the second half of the d^2P/dt^2 waveform.

c) V_{max}.

This term was computed by extrapolating a graph of V_{CE} (dP/dt/developed LVP) *vs* developed LVP, to zero developed LVP. The extrapolation was performed by a computer regression analysis which fitted the best line of the form $Y = A + B\ e^{-Ct}$ to the data (Urschel *et al*, 1980). The intercept on the Y axis is equal to A+B. The best line was inspected visually before approval. Due to the large degree of error introduced by data measured at low pressure values, data below 5mmHg developed LVP were excluded.

d) APD, ARPD and FARPD.

APD was computed using equation (6) shown above. This index is a measure of the area under the graph of dP/dt against time, between the points of minimum LVP and maximum LVP, (Fig.3.5b). This index has units of mmHg/sec.

ARPD was computed using equation (7) shown above. This index is the area under a plot of d^2P/dt^2 against time, between the point of lowest dP/dt and maximal dP/dt (Fig.3.5c) This index has the units of mmHg sec^2.

FARPD was computed using equation (8) shown above. Graphically, this index is similar to ARPD, the difference being that the time scale of the plot has

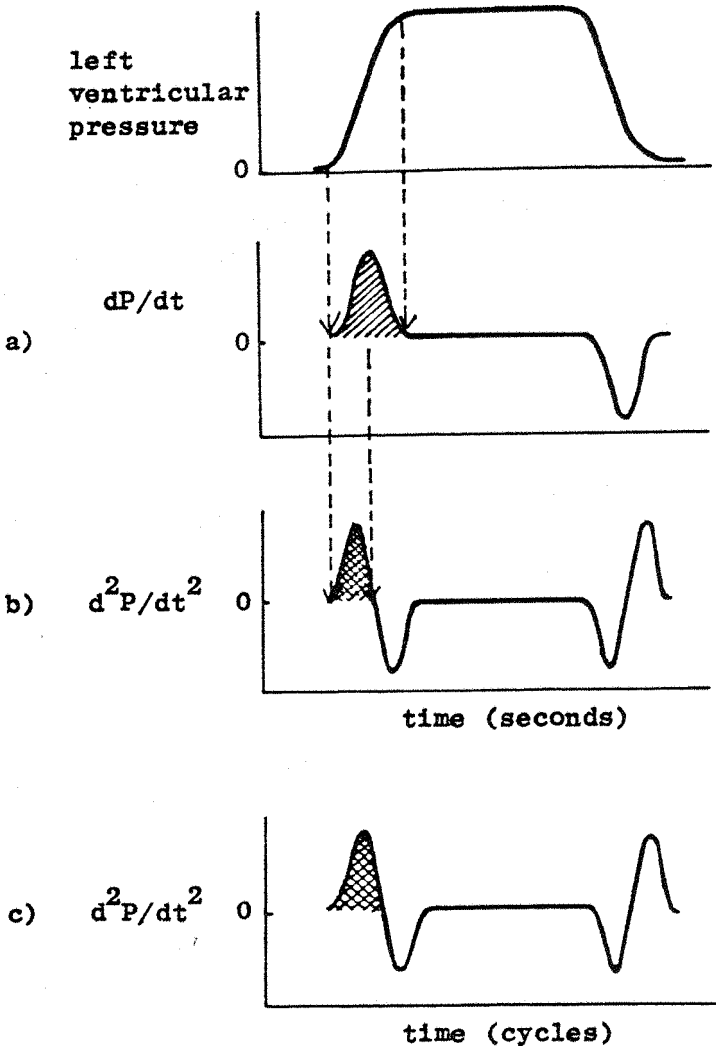


Fig. 3.5
The evaluation of indices of myocardial contractility derived from thermodynamic principles:

a) APD - Energy-averaged power density.
b) ARPD - Energy-averaged rate of power density generation.
c) FARPD - Frequency compensated ARPD.

These indices are evaluated as the area shaded.

been "normalised" using a term derived from the duration of the relevant cardiac cycle (Fig.3.5d). This index has the units of mmHg/cycle^2. FARPD was not computed using the Fourier series of the LVP waveform, as described by Bloomfield et al, (1979), but was computed using the same method of digital integration employed to compute APD and ARPD. The errors involved in the digital integration were minimised by the use of Simpson's rule for integration (Bostock & Chandler, 1978).

3.3.2 The measurement of contractile indices during administration of various inotropic drugs.

Female Wistar albino rats weighing 300-350g were anaesthetised with pentobarbitone and prepared for the measurement of contractile indices using the method described in chapter 2. Drugs were infused into the femoral vein (0.9ml/min) for 5 minutes, at the end of which a measurement was taken. A suitable period of recovery, usually 15mins was allowed before the next drug was applied. The positive inotropic substances adrenaline and calcium ions and the negative inotropic substances carbachol and Verapamil, were administered in the concentrations given below:-

Adrenaline - 65 $\mu g\ kg^{-1}\ min^{-1}$
Carbachol - 32 $\mu g\ kg^{-1}\ min^{-1}$
$CaCl_2$ - 14 $mg\ kg^{-1}\ min^{-1}$
Verapamil - 55 $\mu g\ kg^{-1}\ min^{-1}$

3.3.3 The measurement of contractile indices during graded dobutamine infusions.

Female Wistar albino rats of 300-350g were anaesthetised with pentobarbitone (Sagatal 60mg/Kg, I.P.) and prepared for the measurement of contractile indices using the method described in chapter 2. In addition a cannula was inserted into the jugular vein and advanced until a mark 8.5cm from the tip of the cannula was level with the rats nose; preliminary studies showed that the cannula tip was then lying level with the right atrium. This cannula was used to measure central venous pressure (CVP). Dobutamine was infused via the femoral vein for a five minute period, at the end of which measurements were taken. This drug was infused in doses of 2, 4 and 8 $\mu g\ kg^{-1}\ min^{-1}$. A suitable recovery period, usually 15 min, was allowed between infusions, at the end of which a control measurement was taken.

3.3.4 The measurement of contractile indices during changes in the preload on the heart.

Rats were prepared for the measurement of contractile indices and CVP using the method described in section 3.3.3. The preload on the heart was decreased by the removal of blood from the femoral vein into a heparinised syringe. Blood was removed in 1ml aliquots until 3ml had been removed in total. A measurement was taken 30 seconds after the removal of each 1ml. The preload on the heart was then increased by the replacement of the 3ml of blood in aliquots of 1ml. Measurements were taken 30 seconds after the replacement of each 1ml.

3.3.5 The measurement of contractile indices during changes in the afterload on the heart.

Rats were prepared for the measurement of contractile indices using the method described in section 3.3.3. The afterload on the heart was increased by the infusion of phenylephrine via the femoral vein for a 5 minute period, at the end of which measurements were taken. The phenylephrine was infused in doses of 1.25, 2.5 and 5 mg $kg^{-1}min^{-1}$. A period of recovery was allowed after each infusion, usually 10 mins., at the end of which a control measurement was taken.

3.4 RESULTS.

3.4.1 Control values of contractile indices in the rat.

The basal levels of the indices of contractility (given below as mean ± SEM for 36 rats) were:-

dP/dt_{max}	= 7843	+/- 292	mmHg/sec
d^2P/dt^2_{max}	= 1034	+/- 47	$x10^3$ mmHg/sec^2
APD	= 5662	+/- 198	mmHg/sec
ARPD	= 7202	+/- 373	$x10^3$ mmHg/sec^2
FARPD	= 14035	+/- 844	mmHg/cycle^2
V_{max}	= 32.5	+/- 1.6	sec^{-1}
$-dP/dt_{max}$	= 8286	+/- 401	mmHg/sec
$-d^2P/dt^2_{max}$	= 1570	+/- 102	$x10^3$ mmHg/sec^2

3.4.2 The effect of various inotropic drugs on indices on contractility.

Changes in these indices after treatment with positive and negative inotropes are shown in Fig.3.6.

i) The saline infusion, which was given to measure the effect of the infusion itself, caused significant changes in the two indices of relaxation; $-dP/dt_{max}$ became more negative by +8.1 +/- 1.1% (n=6, P<.01) and $-d^2P/dt^2_{max}$ by +12.1 +/- 3.2% (n=6, P<.05). The other indices of contractility were not significantly changed by infusion of saline.

ii) The infusion of adrenaline produced significant increases in most of the indices. The d^2P/dt^2_{max} showed the largest increase, by 173 +/- 45%, (n=6, P<.05), followed closely by ARPD. The dP/dt_{max} increased by 88 +/- 22%, (n=6, P<.05). The relaxation indices also showed modest but non-significant changes, as did the V_{max}.

iii) The infusion of calcium ions caused significant increases in most indices, the exceptions being the relaxation indices and V_{max}, which all showed small, non-significant, decreases. The FARPD showed the largest increase, by 89 +/-19%, (n=6, P<.01), followed by ARPD and d^2P/dt^2_{max}.

iv) The infusion of carbachol caused decreases in all of the indices, although not all of these changes were significant. The largest decrease was in the $-d^2P/dt^2_{max}$, by 49.1 +/- 11%, n=6, P<.01, followed by significant decreases in ARPD and dP/dt_{max}.

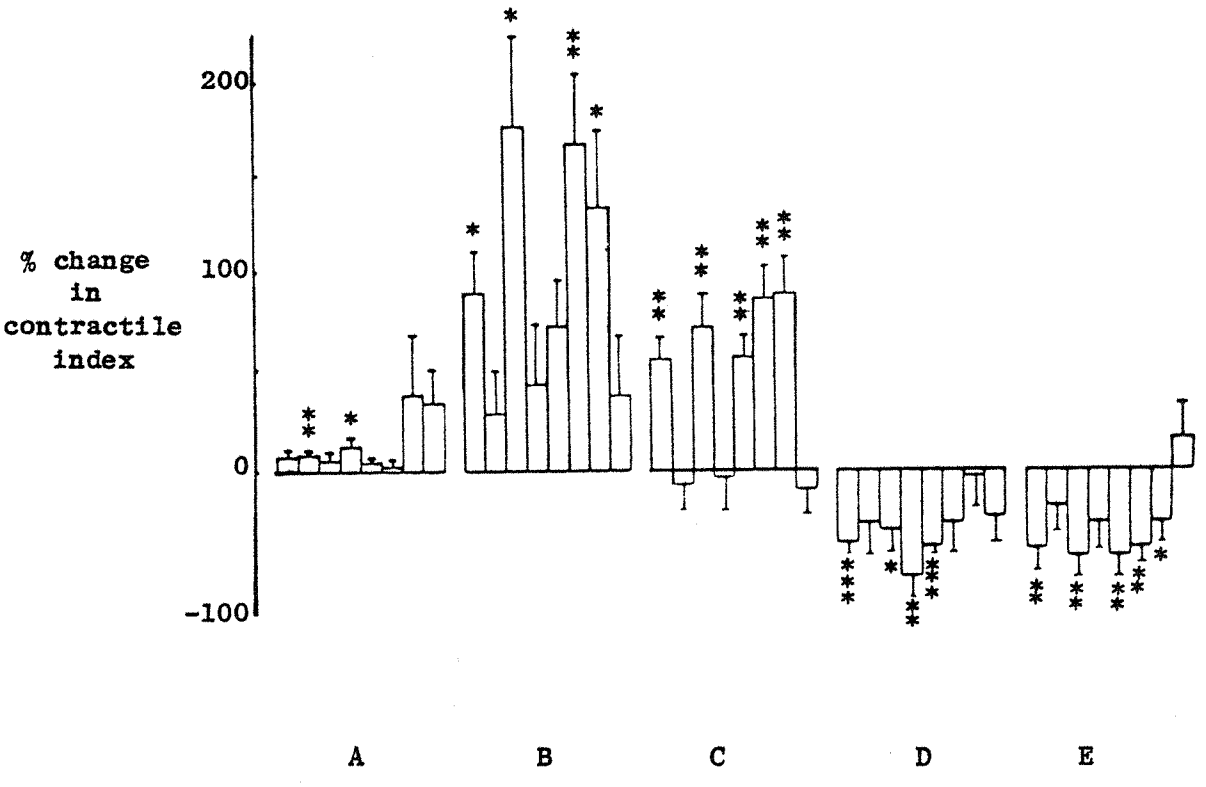


Fig. 3.6
The changes in the contractile indices of rats under the influence of various treatments. The indices are grouped in the following order, from left to right:

1) dP/dt
2) -dP/dt
3) d^2P/dt^2
4) $-d^2P/dt^2$
5) APD
6) ARPD
7) FARPD
8) V_{max}

The drug treatments are indexed as follows:

A) Saline
B) Adrenaline
C) Calcium chloride
D) Carbachol
E) Verapamil.

The values are shown as mean $^{+}$ SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - $P < 0.05$ ** - $P < 0.01$ *** - $P < 0.001$

FARPD showed the smallest change, only -1.0 +/- 15%, (n=6, P>.05).

v) The infusion of verapamil caused decreases in all of the indices except V_{max}. The changes in dP/dt_{max}, d^2P/dt^2_{max}, APD and ARPD were all statistically significant and varied within only 5% of each other (the largest was APD which changed by -41.5 +/- 10%, n=6, P<.05). FARPD decreased significantly although by a smaller percentage.

The changes which occurred in heart rate and mean arterial pressure during the infusion of the drugs are shown in Fig.3.7. Heart rate was significantly increased by adrenaline, but not calcium ions, and significantly decreased by both carbachol and verapamil. Calcium infusion was the only treatment which did not alter blood pressure significantly.

3.4.3 The effect of graded inotropic stimulation with dobutamine on indices of contractility.

The percentage increase in each index plotted against the dose of dobutamine administered (Fig.3.8), and the slope of the relationship was determined by best line linear regression analysis. The slopes (% increase per 10 µg dobutamine) are given below in order of sensitivity to dobutamine i.e. the larger the slope, the greater the sensitivity of the index to stimulation by dobutamine:-

Index of contractility	% change per 10µg dobutamine (n=6 rats)	Coefficient of correlation (r)
ARPD	183	0.992
d^2P/dt^2_{max}	154	0.983
FARPD	142	0.940
APD	81	0.997
dP/dt_{max}	76	0.993
V_{max}	23	0.887

The changes in heart rate, mean arterial pressure and central venous pressure are shown in Fig.3.9. The arterial pressure was not significantly changed by the administration of dobutamine, but the heart rate was significantly increased by the higher doses of dobutamine.

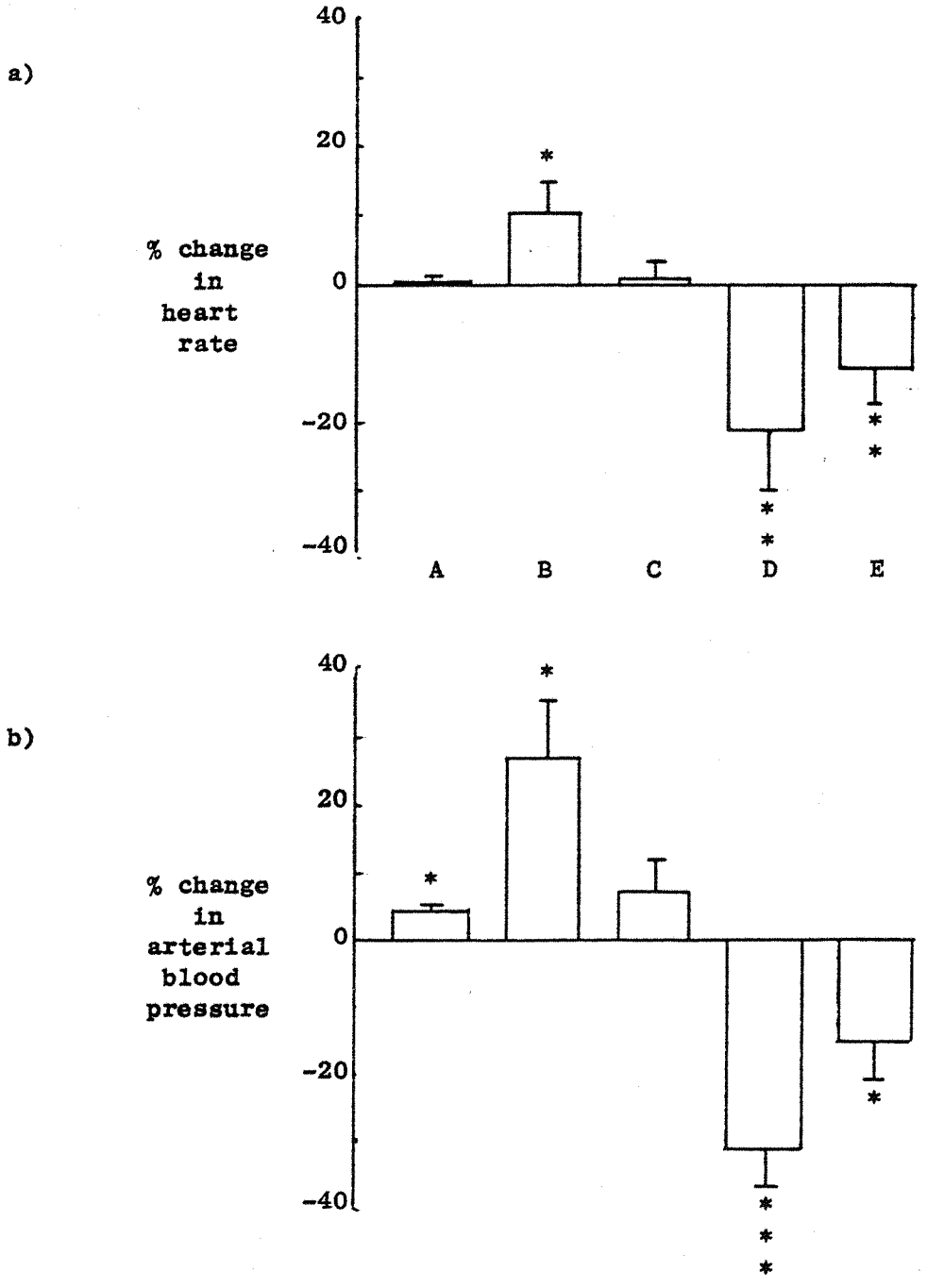


Fig. 3.7
The changes in
a) heart rate,
b) arterial blood pressure,
caused by the treatments listed below:

A)	saline	
B)	adrenaline	(65μg kg^{-1} min^{-1})
C)	calcium ions	(32μg kg^{-1} min^{-1})
D)	Carbachol	(14mg kg^{-1} min^{-1})
E)	verapamil	(55μg kg^{-1} min^{-1})

The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - $P < 0.05$ ** - $P < 0.01$ *** - $P < 0.001$

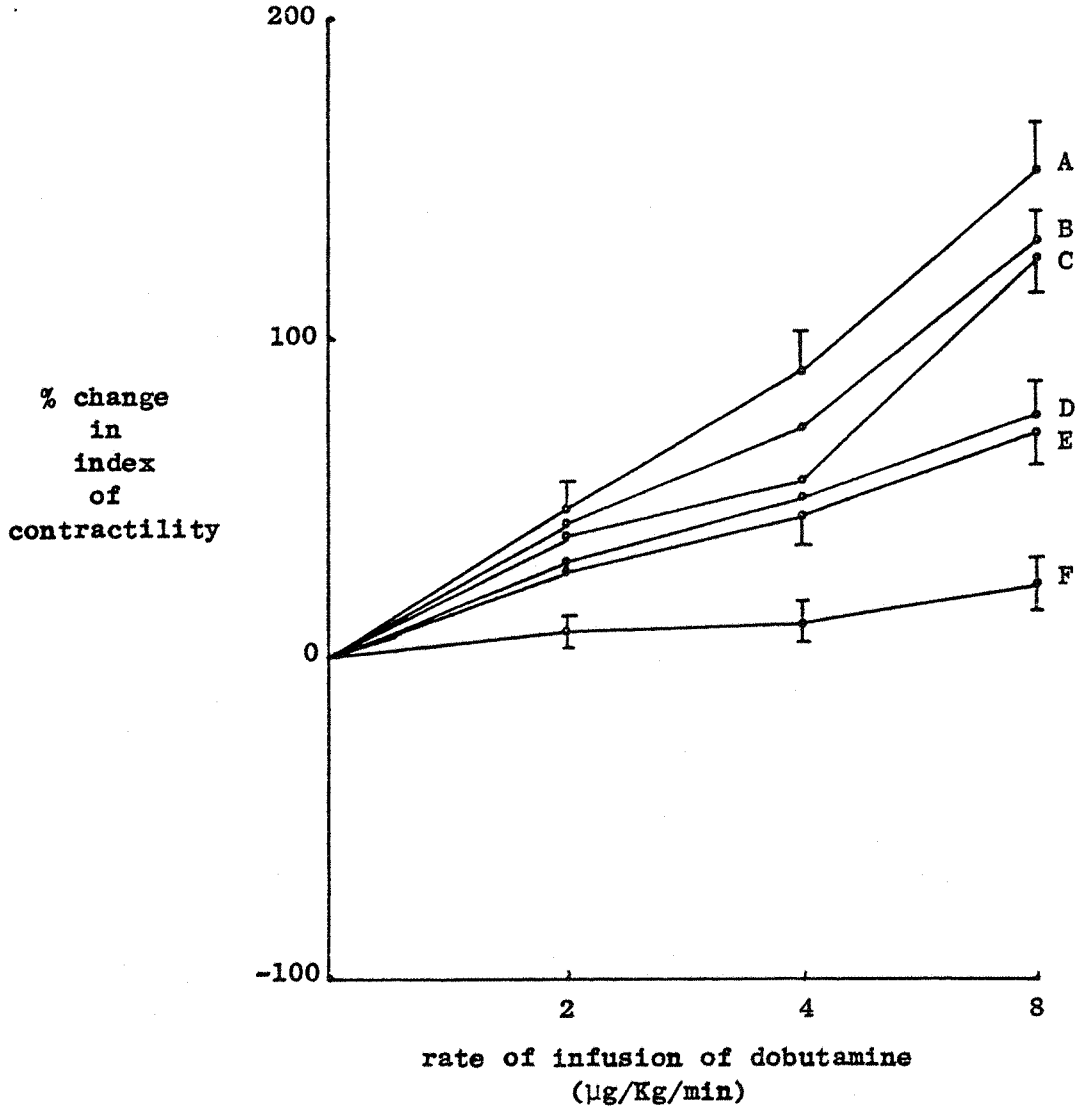


Fig. 3.8
The changes in the indices of contractility due to various rates of infusion of dobutamine. The values are shown as mean ± SEM where possible, otherwise as mean, where n=6 observations per point. The indices are indexed as follows:-

A) ARPD
B) d^2P/dt^2_{max}
C) FARPD
D) APD
E) dP/dt_{max}
F) V_{max}

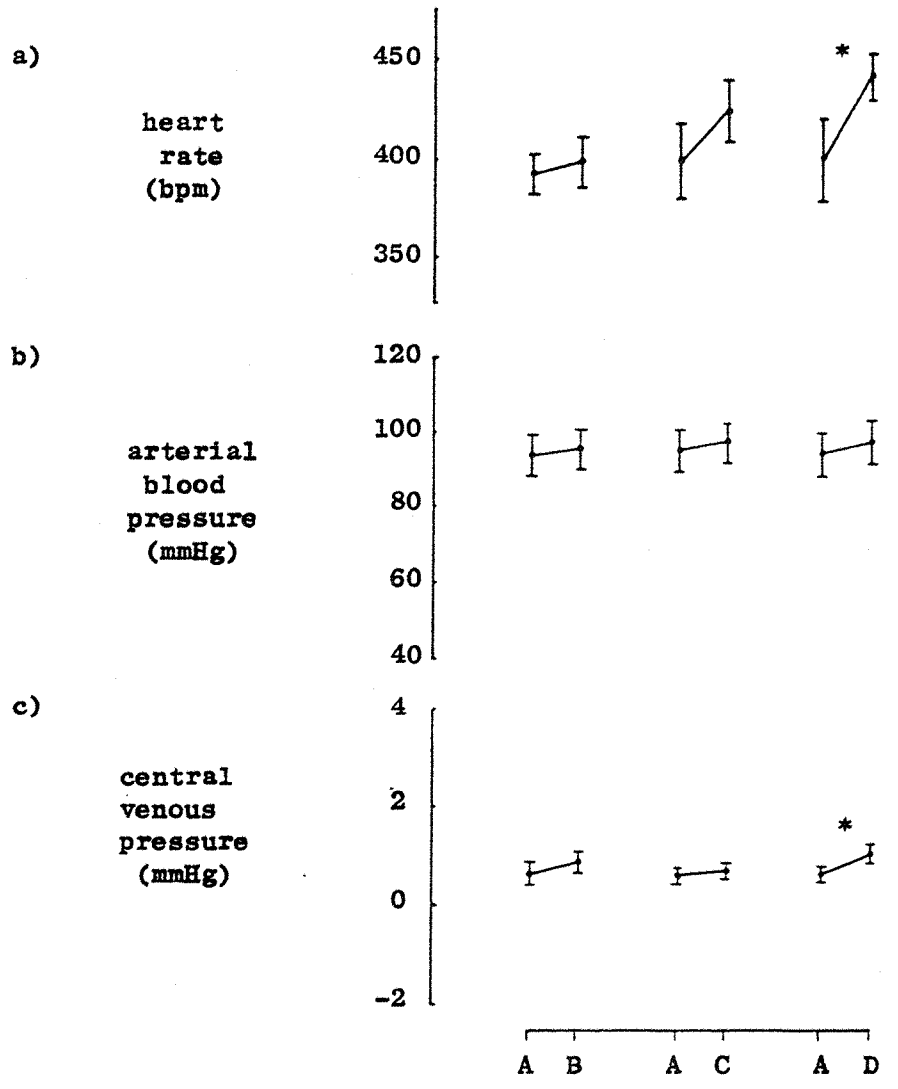


Fig. 3.9
The changes in

a) heart rate,
b) arterial blood pressure,
c) central venous pressure,

after the infusion of graded doses of dobutamine.
The treatments indexed above are:-

A) control measurement
B) infusion of dobutamine (2μg kg^{-1} min^{-1})
C) " 4
D) " 8

The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - $P < 0.05$ ** - $P < 0.01$ *** - $P < 0.001$

3.4.4 The effect of changes in the preload on the heart on indices of contractility.

The percentage change in each index was plotted against the decrease in blood volume (an indicator of the change in preload, Fig.3.10). The slope of the relationship was determined by best line linear regression analysis. The slopes (% change in the index per mmHg CVP) are given below in order of sensitivity to preload i.e. the larger the slope, the greater the dependence on preload.

Index of contractility	% change per mmHg of CVP (n=6 rats)	Coefficient of correlation (r)
ARPD	-24.8	0.994
d^2P/dt^2_{max}	-26.5	0.995
FARPD	-28.7	0.976
dP/dt_{max}	-34.7	0.998
APD	-34.7	0.999
V_{max}	+15.1	0.751
$-dP/dt_{max}$	-38.9	0.992
$-d^2P/dt^2_{max}$	-45.5	0.994

The changes in heart rate, mean arterial pressure and central venous pressure are shown in Fig.3.11.

3.4.5 The effect of changes in the afterload on the heart on indices of contractility.

The percentage change in each index was plotted against the increase in mean arterial blood pressure, an indicator of the afterload (Fig.3.12). The slope of the relationship of the indices to changes in pressure was determined by best line linear regression analysis (in this case the results fron the two lowest doses of phenylephrine were used to calculate the slope). The slopes are given below in order of sensitivity to afterload i.e. the larger the slope, the greater the dependence on afterload.

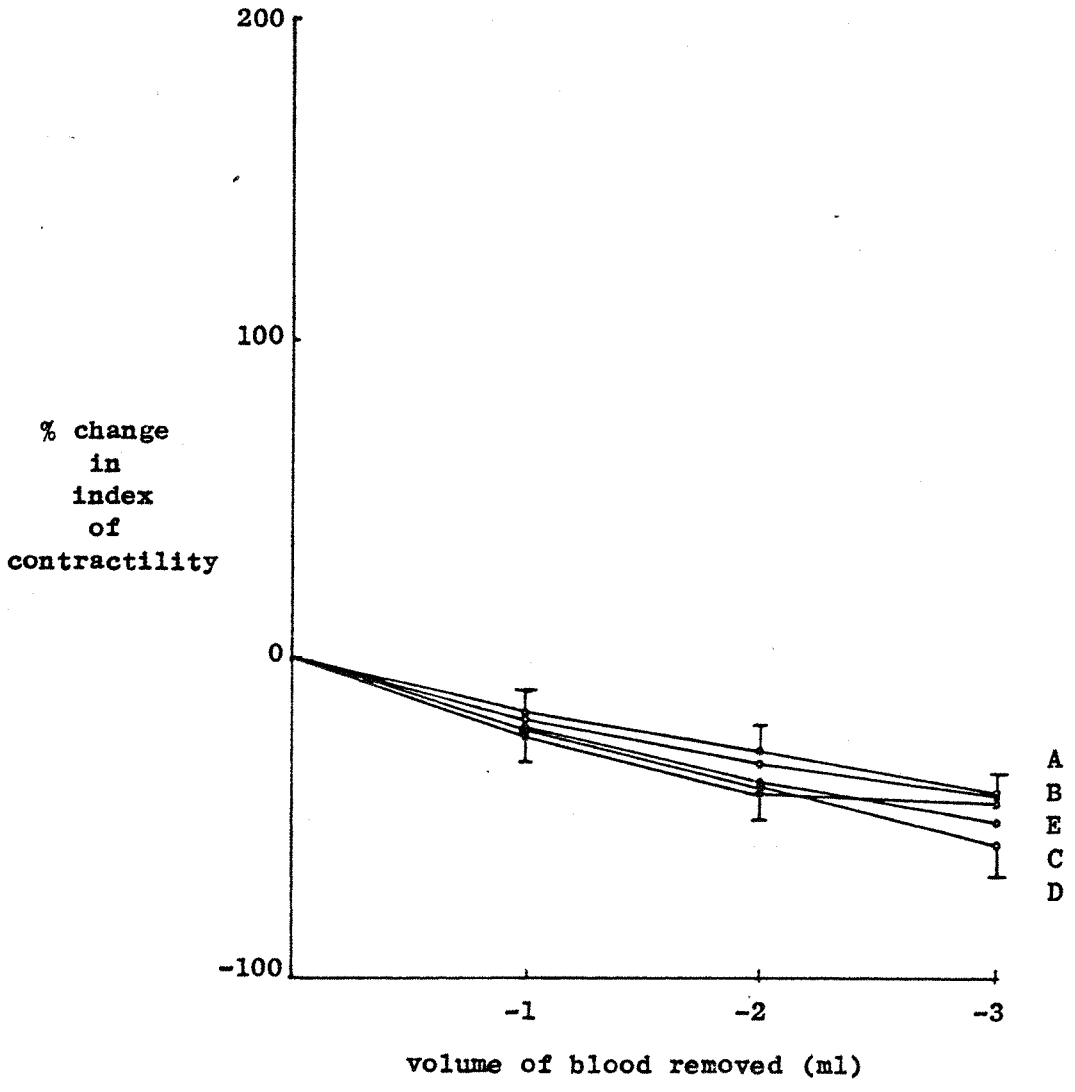


Fig. 3.10

The changes in the indices of contractility due to graded decreases in blood volume. The values are shown as mean ± SEM where possible, otherwise as the mean, where n=6 observations per point. The indices are indexed as follows:-

A) ARPD
B) d^2P/dt^2_{max}
C) FARPD
D) APD
E) dP/dt_{max}
F) V_{max}

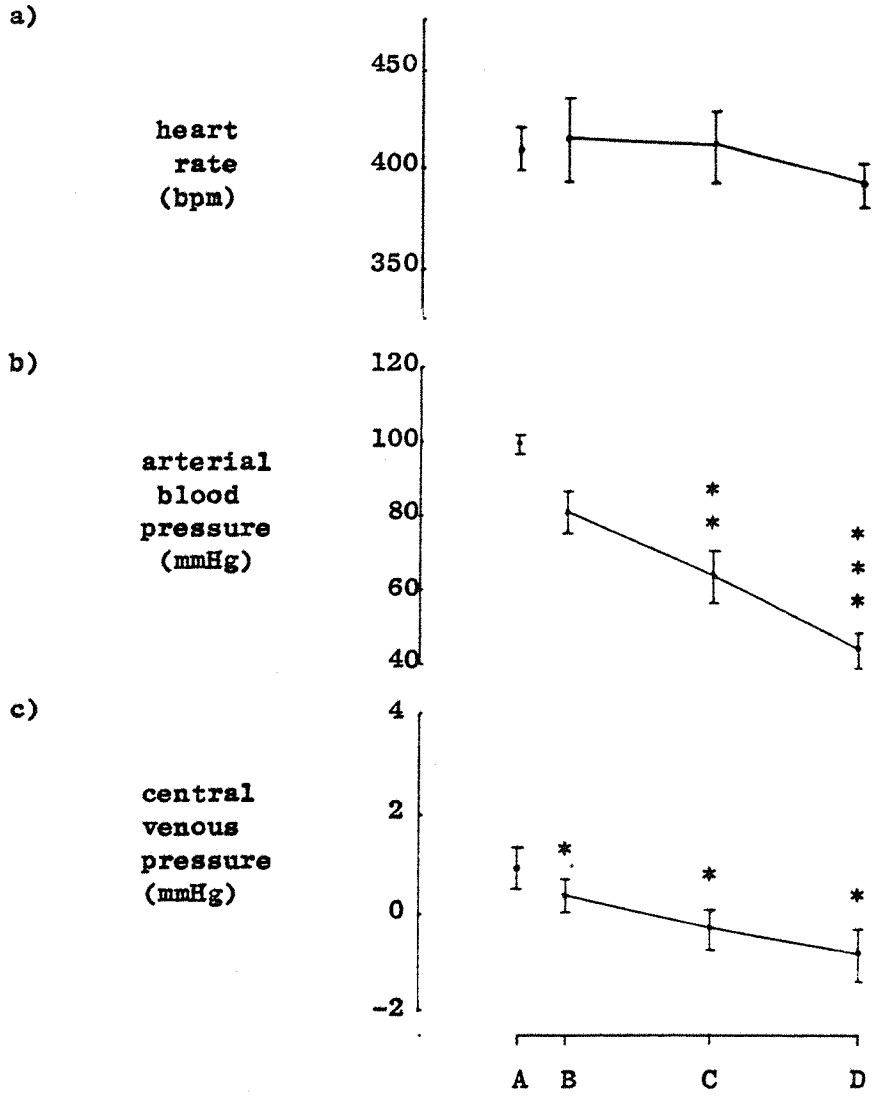


Fig. 3.11
The changes in

a) heart rate,
b) arterial blood pressure,
c) central venous pressure,

after the graded decreases in blood volume. The treatments indexed above are:-

A) control measurement
B) 1ml decrease in blood volume
C) 2 "
D) 3 "

The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - $P < 0.05$ ** - $P < 0.01$ *** - $P < 0.001$

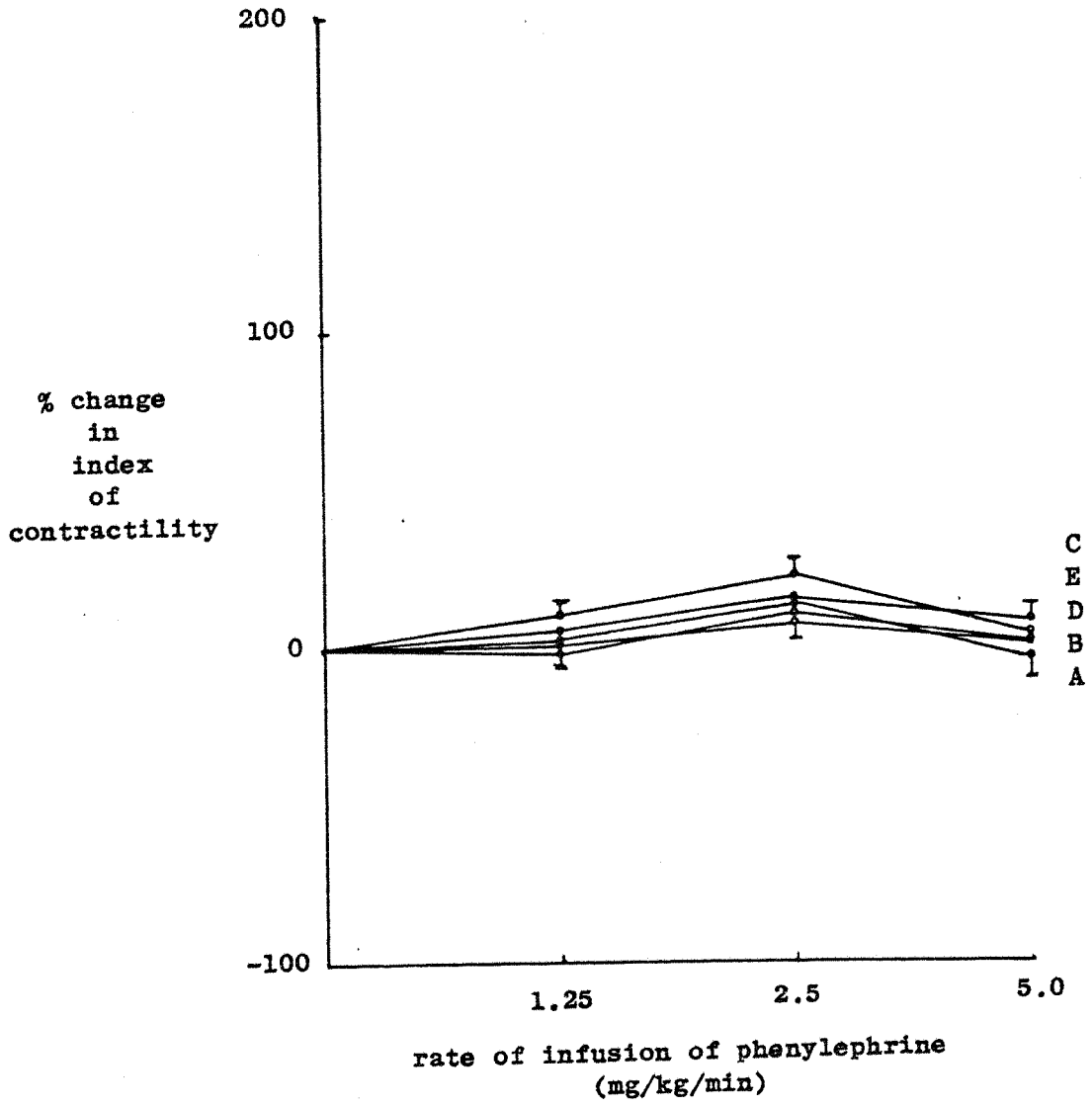


Fig. 3.12
The changes in the indices of contractility due to various rates of infusion of phenylephrine. The values are shown as mean ± SEM where possible, otherwise as mean, where n=6 observations per point. The indices are indexed as follows:-

A) ARPD
B) d^2P/dt^2_{max}
C) FARPD
D) APD
E) dP/dt_{max}
F) V_{max}

Index of contractility	% change per 10mmHg of MAP (n=6 rats)	Coefficient of correlation (r)
ARPD	5.6	0.826
d^2P/dt^2_{max}	7.8	0.741
APD	10.7	0.881
dP/dt_{max}	12.9	0.904
FARPD	17.9	0.995
$-dP/dt_{max}$	7.4	0.957
$-d^2P/dt^2_{max}$	9.7	0.960

No value is given for V_{max} because the index increased at the lower doses of phenylephrine, then decreased at the higher doses; none of the changes in V_{max} was statistically significant.

The changes in the heart rate, mean arterial pressure and central venous pressure are shown in Fig.3.13.

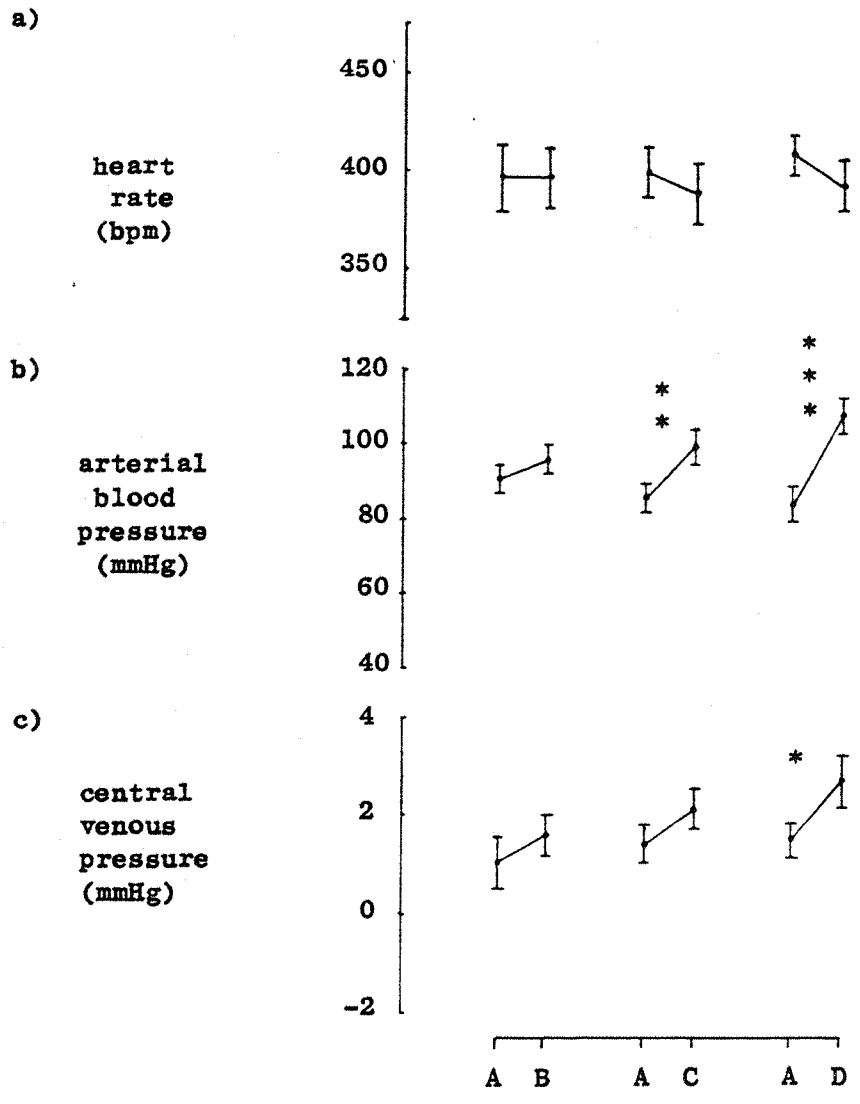


Fig. 3.13
The changes in
a) heart rate,
b) arterial blood pressure,
c) central venous pressure,
after the infusion of graded doses of phenylephrine
The treatments indexed above are:-
A) control measurement
B) infusion of phenylephrine (1.25μg kg-1 min-1)
C) " 2.5
D) " 5.0
The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.
* - $P < 0.05$ ** - $P < 0.01$ *** - $P < 0.001$

3.5 DISCUSSION.

These experiments were performed to investigate the usefulness of various indices of contractility in the anaesthetised rat. Of the indices measured, only dP/dt_{max} has previously been measured accurately in the the rat (see section 2.5). As dP/dt_{max} is much larger in the rat than in other larger species studied, it is doubtful whether there is any benefit to be obtained from comparing values obtained in the current study with values obtained from larger species.

In the present study, the rat preparation was treated with substances which are known to cause changes in contractility. Adrenaline is known to increase heart rate, enhance myocardial contractility and increase the peripheral resistance (Bowman & Rand, 1982). In the present study, adrenaline caused significant increases in heart rate and mean arterial blood pressure.

Carbachol is a potent analogue of acetylcholine, the neuro-transmitter present in parasympathetic nerve terminals. The effects of acetyl-choline on the cardiovascular system are a decrease in heart rate and myocardial contractility and a fall in blood pressure. Such changes were observed in these experiments.

The role of calcium ions in the contractile process are described in section 3.2.1. In this experiment calcium chloride was infused into the whole animal. The short-term effects of calcium ion infusion include increased heart rate, increased contractility and peripheral vasoconstriction, via a direct action on smooth muscle cells (Bowman & Rand, 1982).

The effects of verapamil on the heart include impairment of myocardial contractility and action potential generation, by blocking movement of calcium ions across cardiac muscle cell membranes (Kohlhardt *et al*, 1971). When infused into the whole animal, verapamil also causes the relaxation of smooth muscle.

In the present study, an infusion of saline was given as a control procedure, to determine the response of the various indices of contractility to the vehicle in which the drugs were administered. This procedure caused no statistically significant change in any index of contractility, but did cause statistically significant increases in the relaxation indices.

All of the indices of contractility, except V_{max}, detected the expected inotropic changes induced by the various treatments. V_{max} tended to detect changes induced

by adrenaline and carbachol, but did not detect the expected changes with $CaCl_2$ and Verapamil. The relaxation indices did not generally show consistant changes with any of the various drugs.

The positive inotropic activity of dobutamine is due to its potent B1-adrenoreceptor agonist action. Dobutamine is a particularly useful drug because it has less of a chronotropic effect than other B-adrenoreceptor agonists. This is particularly beneficial to this study, as changes in heart rate may induce reflex increases in contractility. The order of sensitivity to positive inotropic stimulation, as determined by the response to graded doses of dobutamine, shows that ARPD was the most sensitive index, closely followed by d^2P/dt^2_{max} and FARPD. These three indices are all derived from similar information, i.e the rate of acceleration of LVP.

The experiment to determine the dependence of the indices of contractility upon changes in preload, showed that ARPD changed the least with decreasing preload, followed by d^2P/dt^2_{max} and FARPD. V_{max} tended to increase with decreasing preload. The relaxation indices were sensitive to preload. Preload independence is the most controvertial subject within this area of research, because of the doubt surrounding the idea that inotropic state and the Frank - Starling mechanism are inextricably linked by length dependent calcium activation of the contractile apparatus. Lambert et al, (1983), found that dP/dt_{max} and APD were the most preload independent indices, whereas the present study showed that ARPD and d^2P/dt^2_{max} were less preload dependent in the rat than APD and dP/dt_{max}. a finding corroborated by Moreno et al, (1976)

Phenylephrine, an α1-adrenoreceptor agonist, was used to increase the peripheral resistance and elevate mean arterial blood pressure, thus increasing the afterload on the heart. Changes in the afterload on the heart are indicated by changes in arterial blood pressure. The order of sensitivity to afterload, as determined by changes in mean arterial pressure shows that ARPD and d^2P/dt^2_{max} are the least sensitive to afterload. In this study, FARPD, ARPD and d^2P/dt^2_{max} were derived during the isovolumic stage of contraction (i.e. when the aortic valve was closed) and so by definition could not detect changes in afterload. The changes in these indices must therefore reflect the reflex-induced changes in contractile state and heart rate caused by the raising of arterial blood pressure. However, the same cannot be said about the indices APD and dP/dt_{max}. The

dP/dt_{max} usually occurs at the end of isovolumic contraction and in this study the exact time of aortic valve opening could not be determined. Early opening of the aortic valve prevents further increase in the dP/dt and Van Den Bos *et al* (1973) reported that at times of low arterial pressure, aortic occlusion often produced an immediate increase in dP/dt_{max}. Other workers have reported that dP/dt_{max} is afterload independent (Lambert *et al*, 1984).

When trying to interpret these results it must be remembered that the reflexes of the rats were intact and that the changes induced in one parameter could lead to changes in other parameters. It must be realised that unlike other studies on larger species or with *in vitro* preparations, other cardiovascular factors could not be controlled in the present work, indeed it would probably be impossible to produce a graded increase in contractile state without changes in afterload and preload. In these studies, the heart rate, CVP and MAP were all altered significantly at some time during the alterations in preload, afterload and contractile state. None of the indices responded to the largest dose of phenylephrine, but it is not known why.

An observation worthy of note is the difference in response between ARPD and FARPD. In computation they differ only by a factor related to heart rate, but they changed in very similar ways in response to drug treatment and alterations in preload, yet the reflex decrease in heart rate following the elevation in blood pressure led to a large difference in the sensitivity of the two indices to afterload.

An interesting fact to emerge from this study is that the indices derived directly or indirectly from the second derivative of LVP seem to be superior (in terms of sensitivity to contractile state and independence of preload and afterload) to the indices derived from the first derivative (dP/dt). This has been reported previously (Letac *et al*, 1976) but the usefulness of these indices seems to have been limited by the poor reproducibility and high variability also reported by these workers. These problems have not been encountered during the present study, possibly due to the methodology employed in the measurement and calculation of this index.

In vivo, the index of V_{max} derived from the developed pressure was found not to be a useful indicator of contractile state. It showed poor sensitivity to dobutamine and behaved erratically to alterations in preload and afterload. It is generally accepted that the

most important factor in the reproducibility of this index is the method of extrapolation. In this study, a 3rd degree polynomial curve was originally fitted to the plot of V_{CE} *vs* LVP. This produced values which varied little under any of the different experimental conditions. In an effort to try to increase the sensitivity of the index, an exponential curve was fitted to the V_{CE} *vs* LVP plot. This increased the range of the index, but did not significantly improve the behaviour of the index. Similar findings have been reported previously by other workers (Peterson *et al*, 1973; Urschel *et al*, 1980).

CHAPTER 4:
Heart function in the immediate post-burn period.

4.1 GENERAL INTRODUCTION.

The studies described in chapter 1 suggested that the cardiovascular response to burn injury could be understood more easily if there were a fall in cardiac contractility.

The first stage of my investigations covered the development of the techniques and computer software to measure left ventricular pressure accurately in the anaesthetised rat preparation (chapter 2). In the second stage, various indices of myocardial contractility were evaluated in the anaesthetised rat, during control conditions and during drug induced changes in cardiovascular function (chapter 3).

This chapter describes my investigations into cardiac function during the immediate post-burn period. Cardiac function was studied using both *in vivo* and *in vitro* preparations. The *in vivo* study consisted of two parallel experiments: the measurement of cardiac output following a burn injury; and the assessment of ventricular contractility during the two hours immediately following burn injury. This is a particularly important time during the development of burn shock and also one where the use of animal models of burn injury can play an important role.

The other approach I adopted for the assessment of cardiac function was to use an isolated cardiac muscle preparation. This preparation was used to test whether the blood of burned animals contains a transmissable factor which can reduce cardiac function. One important advantage of *in vitro* preparations is that they are not subject to the variability inherent in whole animal experiments, and the data obtained are therefore simpler to interpret.

4.2 LITERATURE REVIEW.

4.2.1 The Effects of Burn Injury on the heart.

Hypovolaemia and/or peripheral vasoconstriction have been proposed as the cause of the rapid fall in cardiac output after a burn injury (see chapter 1). A third hypothesis is that a rapid deterioration in cardiac function initiates or potentiates this fall in cardiac output.

Myocardial insufficiency is often observed during the later stages of progressive circulatory shock, and is probably an integral part of the positive feedback mechanism which leads to circulatory failure and eventually death (Guyton, 1979): a reduction in coronary perfusion leads to a reduced force of contraction which perpetuates the cycle by further reducing cardiac output, which in turn further reduces coronary perfusion. This type of mechanism is present in many types of shock (e.g hypovolaemic shock) and the presence of depressed cardiac function cannot necessarily be considered as proof that myocardial depression initiates this sequence of events.

The possibility of myocardial dysfunction after burn injury was originally implied by the observation that the post-burn restoration of blood volume did not result in a return of cardiac output to pre-burn values (Gilmore, 1957), while the administration of cardiac glycoside in addition to volume replacement, did lead to the restoration of cardiac output to pre-burn values (Fozzard, 1961).

Myocardial depression was first observed after burn injury as a displacement of the left ventricular function curve (Merriam, 1962) and was also suggested by abnormalities in the ECG's of burned patients (Dobson & Warner, 1957). Left ventricular function after burn injury has since been investigated by various workers (Baxter, Cook & Shires, 1966; Moncrief, 1966; Okamoto, Kaye, Coleman & Glaviano 1974; Wolfe & Miller, 1976; Raffa & Trunkey, 1978; Vasilets, Vornovitskii & Khodorov, 1978; Kuzin, Portnoy, Dwortsin & Machulin, 1983; Adams, Baxter & Parker, 1982; Aggarwal, 1983), using a wide range of *in vivo* whole animal preparations and *in vitro* isolated tissue preparations. Myocardial depression generally appears after a large burn injury, but most of these studies were performed several hours after the burn injury, at a time when hypovolaemia may have led to changes in cardiac output and myocardial contractility. The time of onset and cause of myocardial depression after

burn injury have been the subjects of much debate and at present, it is still unclear whether myocardial depression is an initiating factor leading to the fall in cardiac output and the onset of burn shock, or is a consequence of a low cardiac output and the onset of shock.

Opinion is therefore divided over the cause of the post-burn myocardial depression. One school of investigators contends that the onset of myocardial depression is related to the occurrence of coronary vasoconstriction and subsequent myocardial ischaemia, whilst another school of investigators points to the evidence for blood-borne burn toxins / myocardial depressant factors. The major evidence for these two arguments is reviewed below.

Okamoto et al, (1974) studied anaesthetised dogs with scald burns to 35% of their body surface areas. They recorded left ventricular dP/dt_{max} as an index of contractile function and found that it gradually declined after burn injury, even though LVEDP and CVP did not change significantly. At 4 hours post-burn there were significant changes in myocardial metabolism; coronary blood flow and myocardial oxygen uptake had decreased, and although the arterial to venous oxygen extraction ratio remained unchanged, they concluded that the depression in myocardial function was due to ischaemia. The absence of any signs of clinical cardiac failure in the early post burn period, was taken as evidence that the depression in myocardial function was a consequence of the changes in peripheral circulation (i.e intense vasoconstriction) rather than the inhibition of the heart by endogenous circulating substances.

There is, however, considerable evidence from both in vitro, isolated tissue experiments and in vivo, cross circulation experiments, to indicate the existence of endogenous circulating toxins. Rosenthal, (1959) first proposed the existence of such 'burn toxins', and subsequently isolated a glycoprotein from burned human skin, which was lethal when administered to mice, and toxic to HeLa cell cultures. Furthermore, sonication of the purified toxin produced an anti-toxin or competitin which protected against the effects of purified toxin. The glycoprotein depressed an in vitro heart preparation, induced ECG abnormalities and reduced the development of tension (Rosenthal, Hawley & Hakim, 1972). The same workers also found that serum from human burn victims caused a depression in developed tension in an in vitro rat heart papillary muscle preparation (Hakim, Sladek & Rosenthal, 1972). Hakim, (1975) subsequently

reported that digoxin reversed the inhibitory actions of human burn serum on *in vitro* human cardiac papillary muscle.

Similar techniques have been used by a group of Swiss workers to isolate and purify a burn toxin (Allgower, Burri & Cueni, 1968; Stadtler, Allgower, Cueni & Schoenenberger, 1972): they found that sterilised homogenates of burned mouse skin were lethal when injected into normal recipient mice and that the toxicity of the homogenate was maximal when the skin had been burned at 250°C in a water free environment. They also found that homogenates of burned skin from male mice were less toxic than those from female mice and concluded that this was due to the higher collagen content of the skin of males. These workers identified their purified toxin as a high molecular weight lipoprotein which is probably formed by a heat induced polymerisation of naturally occurring membrane components.

A French group has also investigated the physiological properties of serum from human burn victims. Ethanol fractionation of the serum yielded several fractions with toxic activity, including a cardiotoxic fraction (Moati, Sepulchre, Miskulin, Huisman,Moczar, Robert, Monteil & Guilbuad, 1978) and a neurotoxic fraction (Sepulchre, Moati, Miskulin, Moczar,Robert, Monteil & Guilbaud, 1978). The cardiotoxin induced an abnormal ECG and a decreased cardiac output in rats and rabbits, suggesting ischaemia of the cardiac muscle . It also had a direct toxic effect on cultured myocytes (Moati, Auclair, Lechat & Robert, 1979). The cardiotoxin was a small protein with a molecular weight of 8000 Daltons

A Russian group under the direction of Fedorov has also isolated a high molecular weight toxin from burned rabbit skin (Movshev *et al*, 1972). This toxin possessed activity against the cardiovascular system, which was suggested to be due to the weakening of the myocardium (Matvienko & Movshev, 1977).

The presence of endogenous substances which cause deterioration of cardiovascular function after burn injury has also been demonstrated by *in vivo* cross-circulation and transfusion experiments by several workers (Baxter *et al*, 1966; Moncrief, 1966; Banner, Chapman, Jacob, Munday, Pagdin & Philpot, 1980). Raffa & Trunkey, (1978) showed that perfusion of rabbit septal muscle with plasma collected 2 hours post-burn, caused significant contractile dysfunction and ultrastructural changes in the perfused muscle. These workers also ruled

out pH difference, temperature, fibrinogen and other large protein moieties, gram negative organisms and endotoxin, as the cause of the myocardial depression. A problem with many of these reports, however, is that while myocardial depressant factors may be present in the plasma 2 hours after the onset of shock, this does not prove that they were involved in initiating the shock, as it is known that myocardial depression can occur as a consequence of hypovolaemic shock (Lefer et al, 1979).

In recent years many workers have studied cardiac function after burn injury by removing the heart from the animal and assessing its performance in vitro in controlled conditions. Vasilets et al, (1978) investigated the contractile function of rabbit heart papillary muscle excised 10, 60 or 180 minutes after burn injury. They found that the relationship between isometric contractile force and frequency of stimulation, was altered in proportion to the time between burning and excision. They concluded that burn injury has a significant effect on the properties of myocardial cells and that the effect on the frequency-force relationship is similar to that caused by the blocking of membrane calcium channels by the compound D600. Wolfe & Miller (1976) developed a model of burn shock in the conscious guinea pig but were unable to isolate substances capable of inducing myocardial depression using the methods of Rosenthal et al, (1974).

In his excellent review of the current state of research into burn injury, Davies (1982) was sceptical about the importance of burn toxins / myocardial depressant factors in the initiation of burn shock. He favoured hypovolaemia as the sole initiating factor, arguing that previous measurements of plasma volume immediately after burn injury had been unreliable or overestimates of the time required for plasma volume to decrease significantly.

The purpose of the present study was to measure indices of ventricular contractility during the immediate post-burn period in vivo, with emphasis on the time scale and severity of changes in cardiac function after burn injury in relation to the fall in cardiac output and onset of burn shock. The possible involvement of endogenous circulating factors was also investigated in an in vitro heart preparation. These investigations should give valuable information concerning the factors involved in the initiation of burn shock.

4.3 METHODS.

4.3.1 A modified apparatus for the application of burn injury.

The studies of chapter 2 have shown that the temporary disconnection of a catheter from its transducer is likely to cause a deterioration in its frequency-response characteristics. It was therefore necessary to construct a new piece of apparatus, so that a burn injury of controlled dimensions and severity could be applied to the anaesthetised rat, without the need for this disconnection. The rat was laid on a cradle of cords attached to a metal frame and the pressure transducers were secured by clamps attached to the metal corner posts. A burn or mock-burn injury was applied to the rat by placing the cradle in a water-bath and releasing the tension on the supporting cords, thus lowering the dorsal surface of the rat into the water. After 20 seconds, the cradle was placed into the cold water-bath, and 60 seconds later, the supporting cords were re-tensioned. The area of the burn injury could be varied by adjustment of the supporting cords.

4.3.2 The measurement of general cardiovascular function after mock-burn and burn injury.

Female Wistar albino rats weighing 300-350g were anaesthetised with pentobarbitone (Sagatal, 60mg/Kg I.P.), shaved on their dorsal surface, tracheostomised, prepared for impedance cardiography (as described in chapter 1) and a cannula inserted into the femoral artery for the measurement of arterial pressure.

Haematocrit values were not measured during this experiment, to avoid causing changes in blood volume and also to keep experimental conditions similar to those of the parallel experiment (the assessment of ventricular contractility). The coefficient of resistivity (r) required for the calculation of stroke volume, was estimated using haematocrit values obtained from similar experiments performed previously.

Rats were sacrificed at the end of each experiment while still under anaesthesia.

4.3.3 The measurement of indices of myocardial contractility after mock-burn and burn injury.

Female Wistar albino rats weighing 300-350g were anaesthetised with pentobarbitone (Sagatal, 60mg/Kg I.P.), shaved on their dorsal surface, tracheostomised and

then prepared for the measurement of contractile indices (as described in chapter 3).

4.3.4 The isolated guinea pig atria preparation.

Duncan-Hartley guinea pigs of either sex were sacrificed by cervical dislocation and the heart was extracted and placed in cold, oxygenated Krebs-Ringer solution. The atria were isolated, then suspended in a 10ml organ bath in oxygenated Krebs Ringer solution. The temperature of the bath was kept at a constant 36°C by a water circulator and thermostat. The atria were suspended from an isometric force transducer (Grass), which was attached to a micrometer scale manipulator which was used to change (and measure) the length of the atria.

The output from the transducer was differentiated by an electronic circuit (Devices Inc.) and both the tension (T) and dT/dt signals were recorded on a Grass polygraph.

4.3.5 The collection of plasma from burn and mock-burn injured rats.

Female Wistar albino rats weighing 300g were anaesthetised with pentobarbitone (Sagatal, 60mg/Kg, IP) tracheostomised, and the carotid artery cannulated. The rats then received either a burn or mock-burn injury to approximately 40% of total body surface area using the new 'burn cradle'. Five minutes later, the arterial cannula was attached to a syringe pump which withdrew 5-6ml of blood in as short a time as possible (usually 2 mins.) into a heparinised syringe. The blood was centrifuged at 6000 r.p.m for 5 mins and the plasma was separated from the cell fraction. The procedure was identical for both burned and mock-burned rats.

4.3.6 The measurement of PaO_2, $PaCO_2$ and arterial blood pH.

Animals were subjected to a burn or mock-burn injury and small samples of arterial blood (0.15ml) were allowed to flow from an arterial cannula, directly into a blood gas analyser (Radiometer). The values of PaO_2, $PaCO_2$ and pH were displayed immediately. The base excess and plasma bicarbonate were calculated from an alignment nomogram (Siggaard-Andersen, 1963).

4.4 RESULTS

4.4.1 Changes in cardiovascular function after burn or mock-burn injury in the anaesthetised rat.

Experimental measurements were started 30 minutes after the completion of preparative surgery. The row of dashes below represents the time scale of the experiments, from 30 minutes before to 90 minutes after the burn or mock-burn.

x x x x x x x x x x x

-30_____0_____30_____60_____90
(mins)

x = Cardiovascular measurements taken

Measurements were taken at -30, -20, -10, +5, +10, +15, +20, +30, +45, +60, +75, +90 minutes, as indicated on the scale above.

The average area covered by the burn injury was 27.5 ± 1.13% (n=6) of the total body surface area.

The changes in cardiac output are shown in Fig.4.1a. In the mock-burned rats, cardiac output increased until it was 23.6 ± 8.1% above the pre mock-burn value at 45 minutes. The cardiac output remained elevated for a further 30 minutes and by the end of the experiment was 12.7 ± 5% above its pre-burn value. The burn injured rats, however, showed an immediate, progressive fall in cardiac output which had decreased by 33.5 ± 5% after 15 mins ($P<.001$, n=6). The cardiac output remained at this significantly depressed level for the rest of the experiment.

The changes in stroke volume and heart rate are shown in Fig, 4.2. The stroke volume appeared to be the major determinant of the fall in the cardiac output of the burn injured rats. The heart rate of the mock-burned rats showed only small fluctuations from control, pre-mock-burn levels, although these changes were occasionally statistically significant ($P<.05$ by paired 't' test, n=6). In the burn injured rats, heart rate tended to decrease slightly to just below pre-burn levels, but this was not statistically significant.

The changes in mean arterial blood pressure are shown in Fig.4.1b. The mock-burned rats showed a small initial decrease in blood pressure of 5.8 ± 1.6% after 10 mins. ($P<.05$, n=6). By 20 mins. post-mockburn, blood

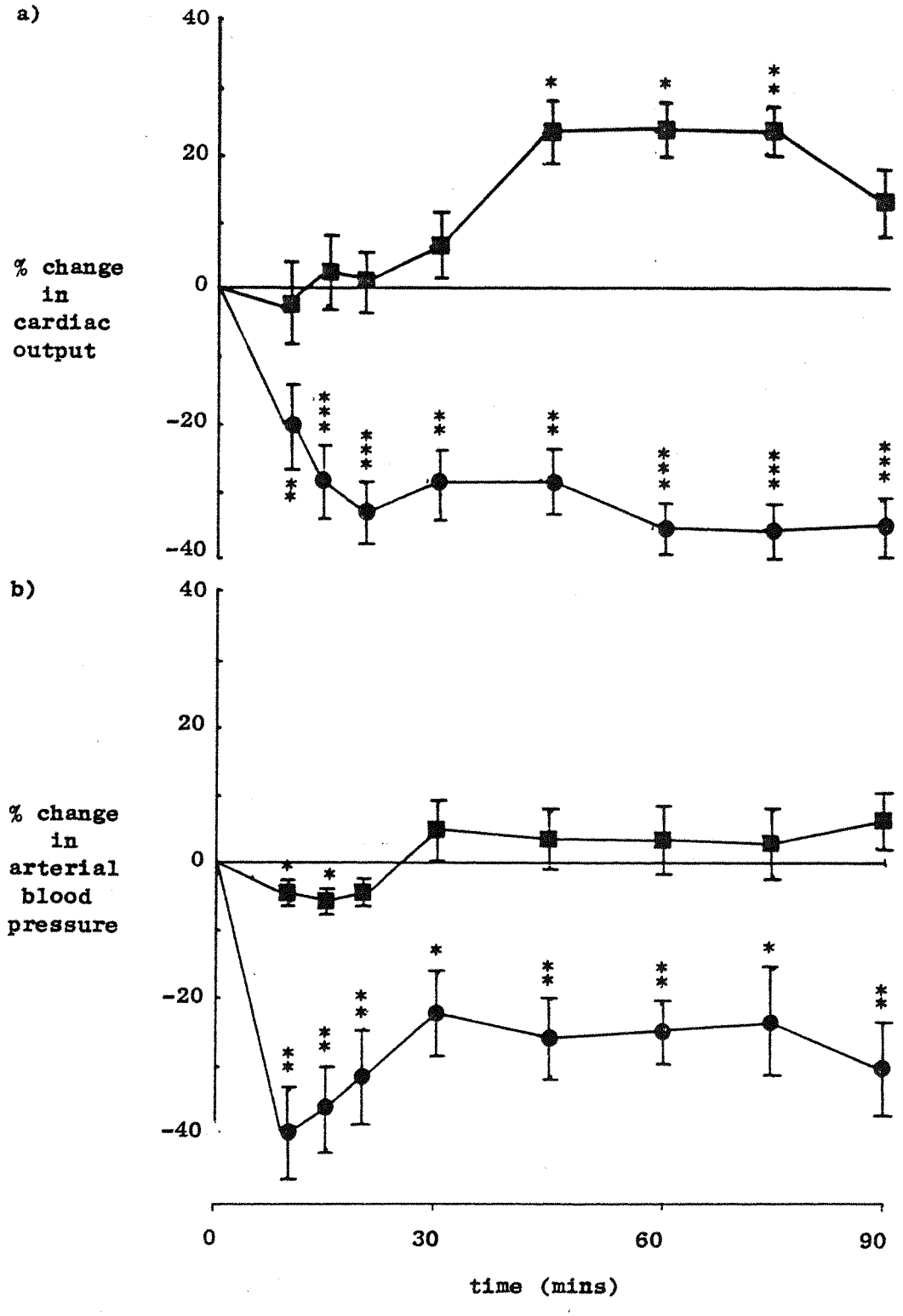


Fig. 4.1

The measurement of cardiovascular function; changes in

a) cardiac output,

b) arterial blood pressure,

after mock-burn and burn injury. The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - P 0.05 ■ - mock-burned rats

** - P 0.01 ● - burn injured rats

*** - P 0.001

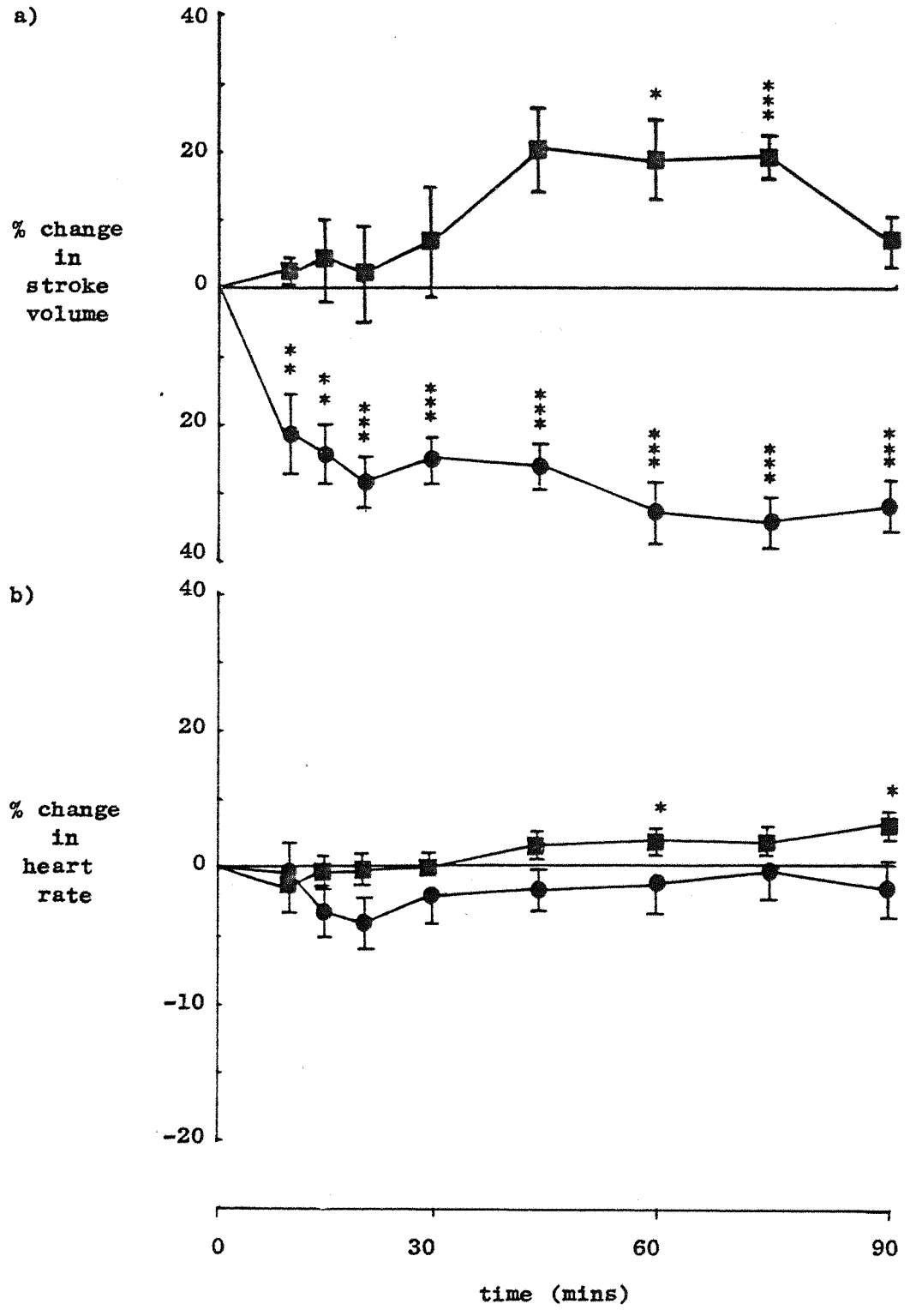


Fig. 4.2
The measurement of cardiovascular function; changes in
a) stroke volume,
b) heart rate,
after mock-burn and burn injury. The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05
** - P < 0.01
*** - P < 0.001

■ - mock-burned rats
● - burn injured rats

pressure returned to control levels and remained thereabouts until the end of the experiment. In burn injured rats, however, the blood pressure fell initially by 40.5 ± 7% (P<0.01, n=6) after 10 mins., recovered slightly to 22.4 ± 6% below the pre-burn value at +30mins. (P<.05, n=6) and then stabilised at this level for the rest of the experiment.

The changes in peripheral conductance are shown in Fig.4.3. The mock-burned rats showed no significant change in conductance, although this did tend to rise above the pre-burn value. The burn injured rats showed an initial increase in conductance of 24.2 ± 10% after 10 mins, although this rise was not statistically significant. The conductance then decreased steadily until 30 mins. post burn and remained a little below control values for the remainder of the experiment. None of the changes in conductance was statistically significant.

4.4.2 Changes in the indices of contractility after burn or mock-burn injury in the anaesthetised rat.

The time course for this experiment was exactly the same as in 4.4.1 above (these two experiments were performed in parallel). Measurements of left ventricular pressure, mean arterial pressure, heart rate and intra-thoracic pressure were taken at -30, -20, -10, +10, +15, +20, +30, +45, +60, +75 and +90 minutes. The following indices of left ventricular function were evaluated from the corrected left ventricular pressure measurements; dP/dt_{max}, $-dP/dt_{max}$, d^2P/dt^2_{max}, $-d^2P/dt^2_{max}$, V_{max} (developed pressure), APD, ARPD and FARPD (as described in chapter 3).

The average area covered by the burn injury in this experiment was 28.0 ± 0.6% (n=6) of the total body surface area.

Changes in the heart rate are shown in Fig.4.4a. There were no significant changes in heart rate after mock-burn or burn injury. In both cases the rate initially decreased slightly, but later increased to just above control levels.

Changes in arterial blood pressure are shown in Fig.4.4b. Mock-burned rats showed no significant changes in blood pressure during this experiment. Burn injured rats, however, showed a significant initial decline in blood pressure, of 26.4 ± 5% at 20 mins. post-burn (P<.01, n=6). The blood pressure then steadily returned to control values and remained at that level for the rest of the experiment.

The indices of ventricular contractility changed

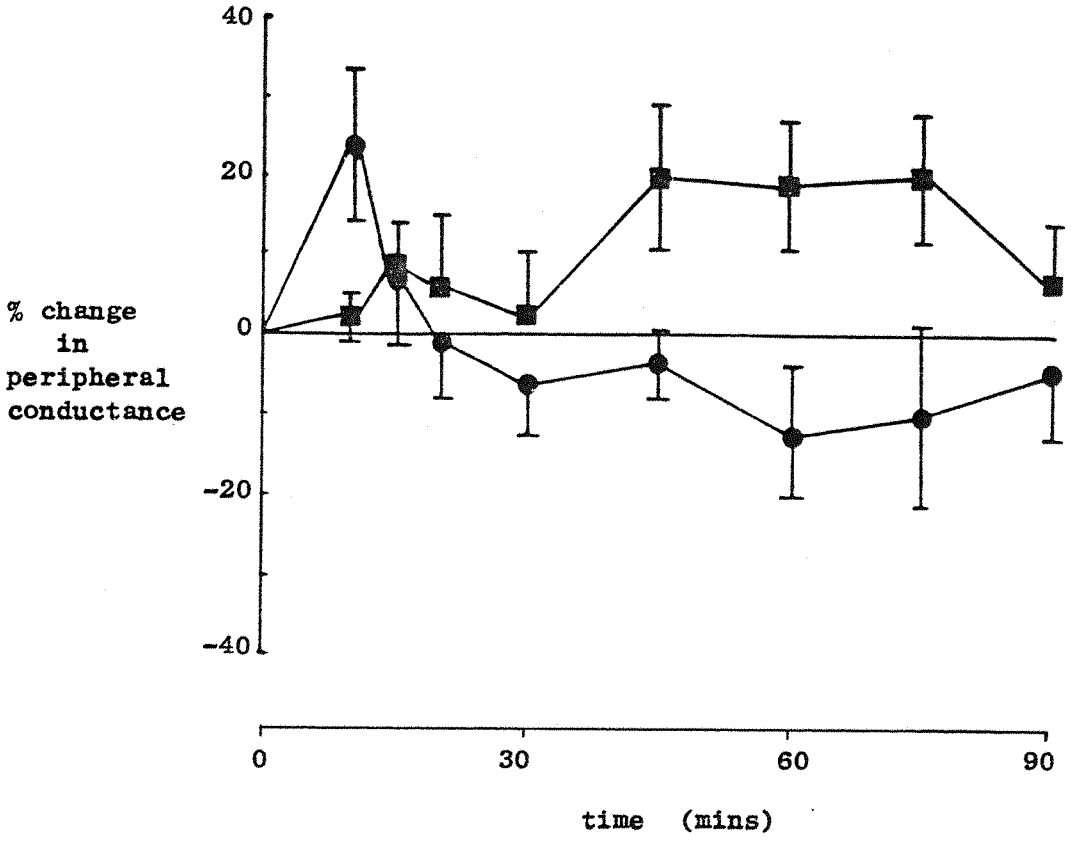


Fig. 4.3
The measurement of cardiovascular function; changes in peripheral conductance after mock-burn and burn injury. The values are shown as mean ± SEM (n= 6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05
** - P < 0.01
*** - P < 0.001

■ - mock-burned rats
● - burn injured rats

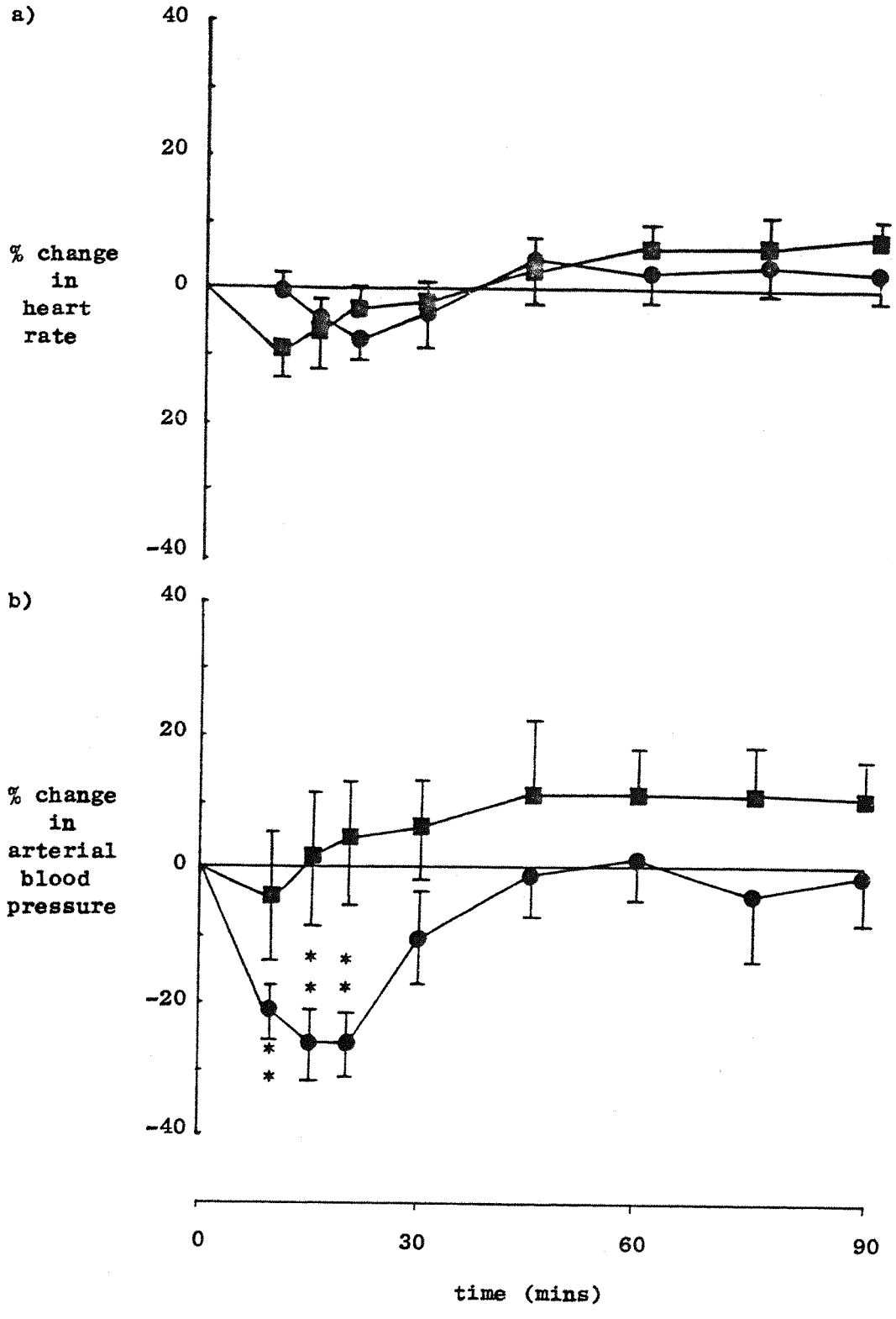


Fig. 4.4

The measurement of contractile indices; the changes in

a) heart rate,

b) arterial blood pressure,

after mock-burn and burn injury. The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05
** - P < 0.01
*** - P < 0.001

■ - mock-burned rats
● - burn injured rats

during the course of the experiment as shown in Figs. 4.5-4.8. Mock-burn injury rarely caused statistically significant changes in the indices of contractility. Most indices tended to stabilise at a level slightly above the pre-mock-burn values. Burn injury, however, caused the following changes in the indices of contractility:

i) dP/dt_{max} decreased significantly during the first 15 mins, by 44.8 + 4%, ($P<.001$, n=6), after which it rose and stabilised at 18.8 $\pm$ 7% below pre-burn values at 90 mins. post-burn ($P<.05$, n=6), as shown in Fig.4.5a.

ii) d^2P/dt^2_{max} decreased significantly during the first 15 mins., by 35.2 $\pm$ 6% ($P<.001$, n=6), but returned towards pre-burn levels by 30 mins. post burn, as shown in Fig.4.5b.

iii) APD decreased significantly during the first 20 mins, by 46.1 $\pm$ 7% ($P<.001$, n=6), then stabilised at a level below, but not significantly different from, pre-burn values for the second half of the experiment, as shown in Fig.4.6a.

iv) ARPD decreased significantly during the first 15 mins, by 34.9 $\pm$ 8% ($P<.01$, n=6), then stabilised at a level which was not significantly different from pre-burn values during the second half of the experiment, as shown in Fig.4.6b.

e) FARPD was not significantly changed by burn injury. This index was slightly depressed between 10 and 20 mins post burn, but then rose above pre-burn levels for most of the remainder of the experiment, as shown in Fig.4.7a.

f) V_{max} did not change significantly from pre-burn values during the duration of the experiment, as shown in Fig.4.7b.

g) $-dP/dt_{max}$ and $-d^2P/dt^2_{max}$ showed very similar changes, with initial significant decreases during the first 15 minutes, then increases back to pre-burn levels by 45 mins and stabilisation at this level during the second half of the experiment, as shown in Fig. 4.8.

The differences between burn and mock-burn groups

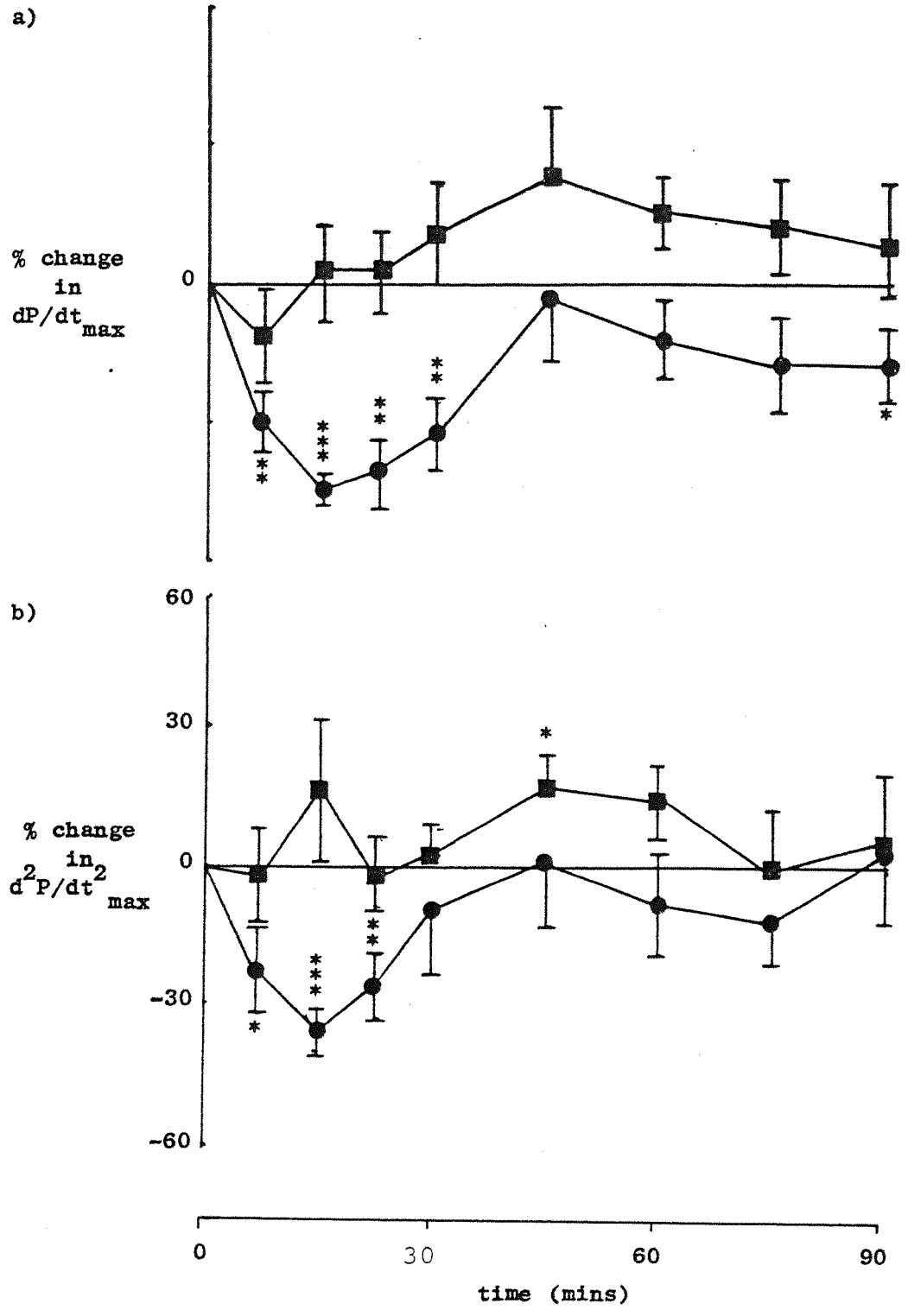


Fig. 4.5

The measurement of contractile indices; the changes in

a) dP/dt_{max}

b) d^2P/dt^2_{max}

after mock-burn and burn injury. The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05

** - P < 0.01

*** - P < 0.001

■ - mock-burned rats

● - burn injured rats

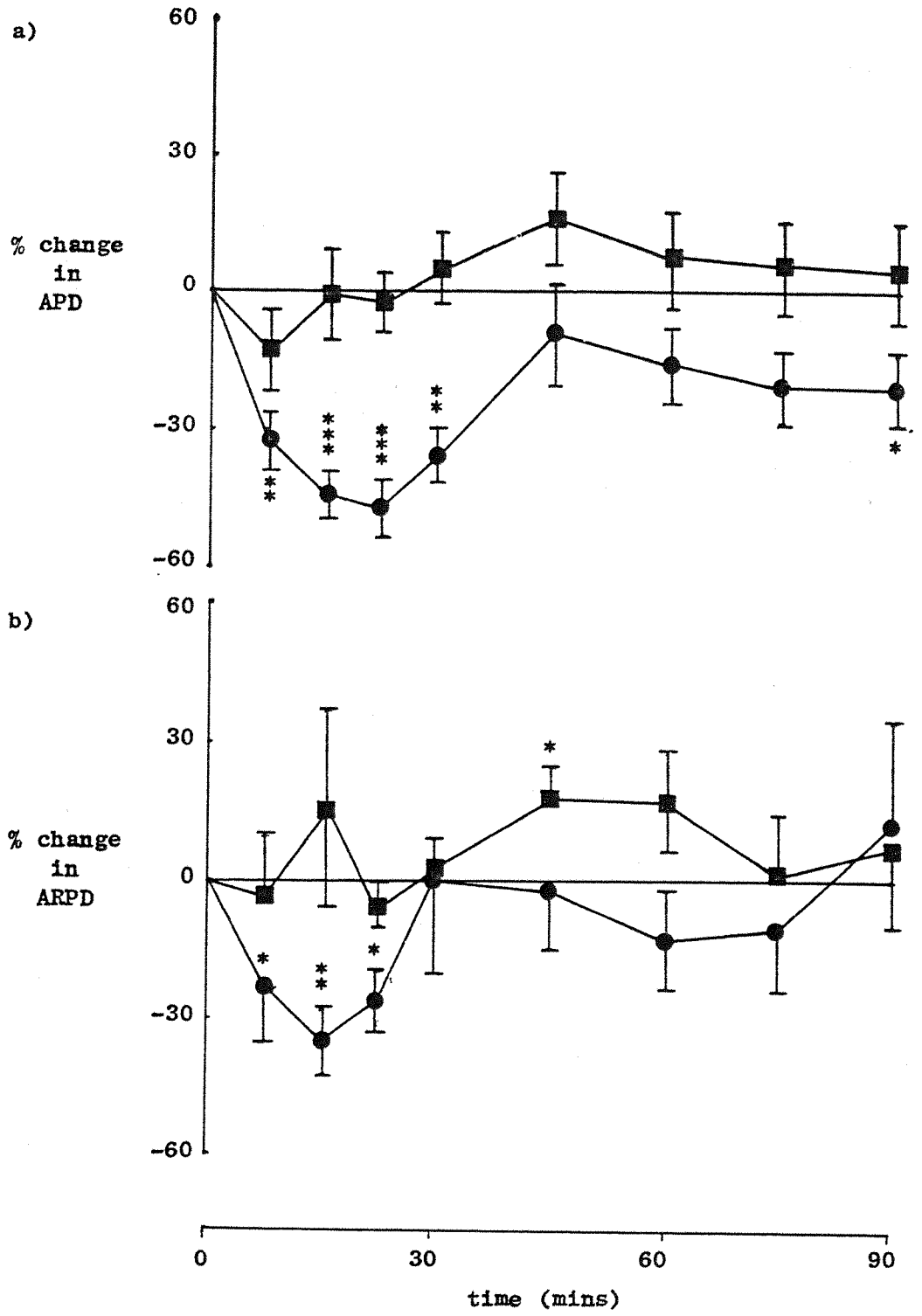


Fig. 4.6

The measurement of contractile indices; the changes in

a) APD

b) ARPD

after mock-burn and burn injury. The values are shown as mean ± SEM (n=6 for all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05

** - P < 0.01

*** - P < 0.001

■ - mock-burned rats

● - burn injured rats

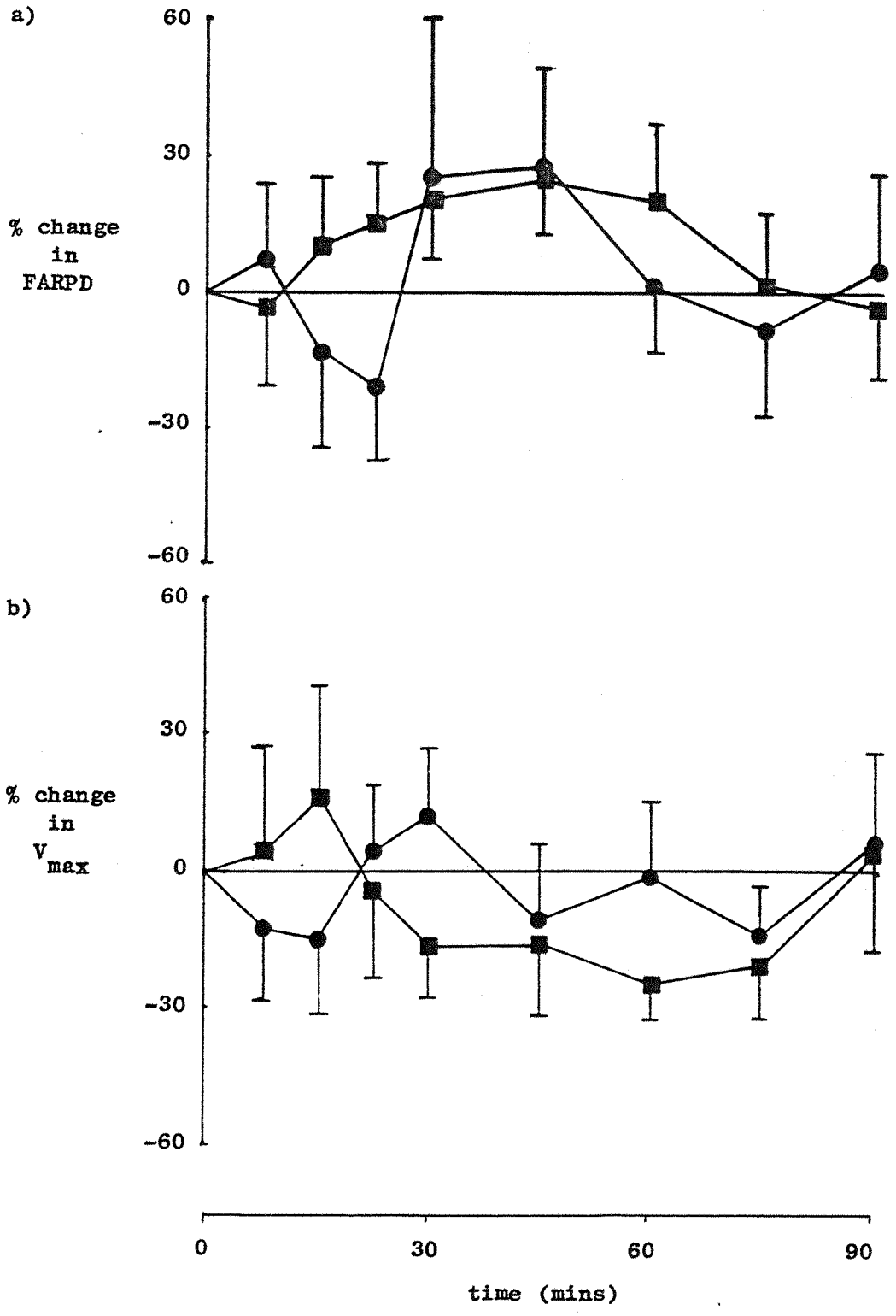


Fig. 4.7
The measurement of contractile indices; the changes in

a) FARPD

b) V_{max}

after mock-burn and burn injury. The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05
** - P < 0.01
*** - P < 0.001

■ - mock-burned rats
● - burn injured rats

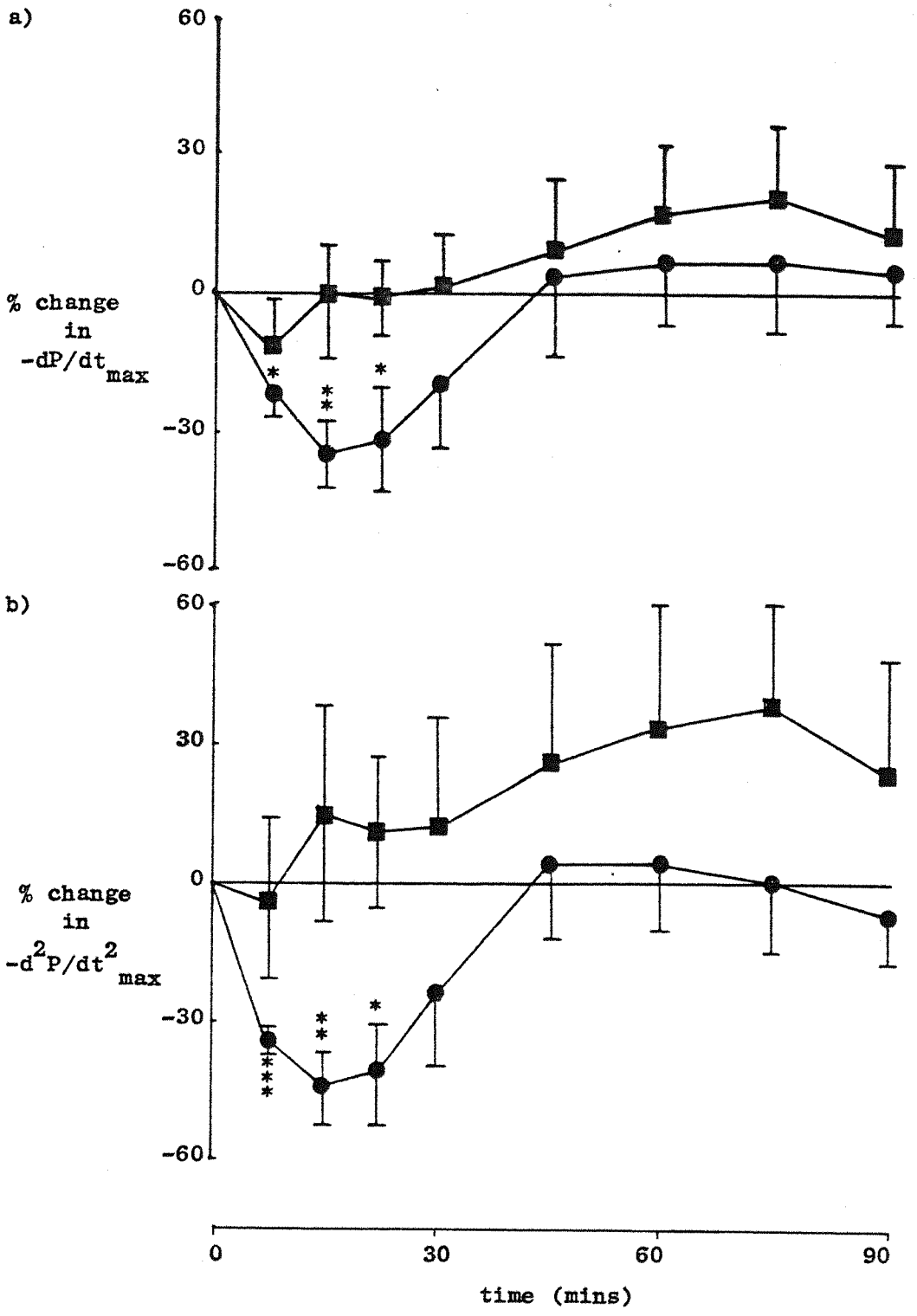


Fig. 4.8

The measurement of contractile indices; the changes in

a) $-dP/dt_{max}$

b) $-d^2P/dt^2_{max}$

after mock-burn and burn injury. The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - P < 0.05

** - P < 0.01

*** - P < 0.001

■ - mock-burned rats

● - burn injured rats

were calculated for each stage of the experiment. The average change in each index during the first 60 mins of the experiment was:-

$$dP/dt_{max} = -35.5 \pm 5.0\%, \quad P < 0.001$$
$$APD = -32.9 \pm 4.8\% \quad P < 0.001$$
$$d^2P/dt^2_{max} = -25.9 \pm 6.2\% \quad P < 0.01$$
$$ARPD = -22.9 \pm 7.4\% \quad P < 0.05$$
$$FARPD = -9.7 \pm 7.8\% \quad N.S.$$
$$V_{max} = +4.5 \pm 9.7\% \quad N.S.$$

$$-d^2P/dt^2_{max} = -38.2 \pm 6.0\% \quad P < 0.001$$
$$-dP/dt_{max} = -19.9 \pm 5.0\% \quad P < 0.01$$

These changes were analysed by unpaired 't' test, (n=6 in all cases).

4.4.3 The effect of temperature on the contractile function of the isolated guinea pig atria preparation.

The thermostat in the water circulator kept variation in bath temperature to within 0.5°C. When the thermostat setting was lowered, the force of contraction of the atria increased, as shown in Fig.4.9a.

4.4.4 The effect of adrenaline, dopamine, histamine and acetyl-choline on the contractile function of the isolated guinea pig atria preparation.

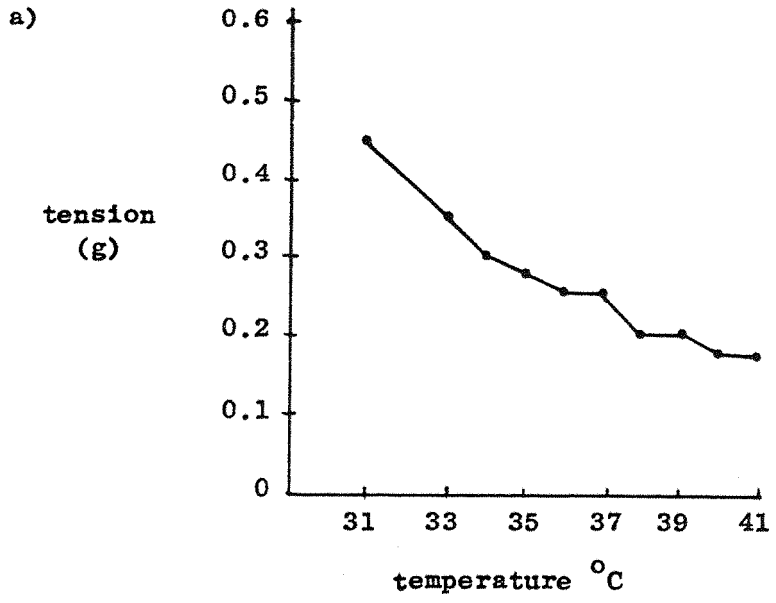
The response of the isolated atria to various chemicals was measured. A typical dose response curve, in this case, to dopamine, is shown in Fig 4.9b. Observed pD_2 values for these responses were:-

Substance		pD_2
Adrenaline	-	5.24
Dopamine	-	4.30
Histamine	-	5.44
Acetyl-Choline	-	7.14

These values indicate that the preparation was responding in the expected way to common pharmacological agents.

4.4.5 The effect of calcium ions on the length-tension relationship of isolated guinea pig atria.

The length-tension relationship for the tissue was obtained by increasing the length of the tissue and recording the tension in the spontaneously contracting



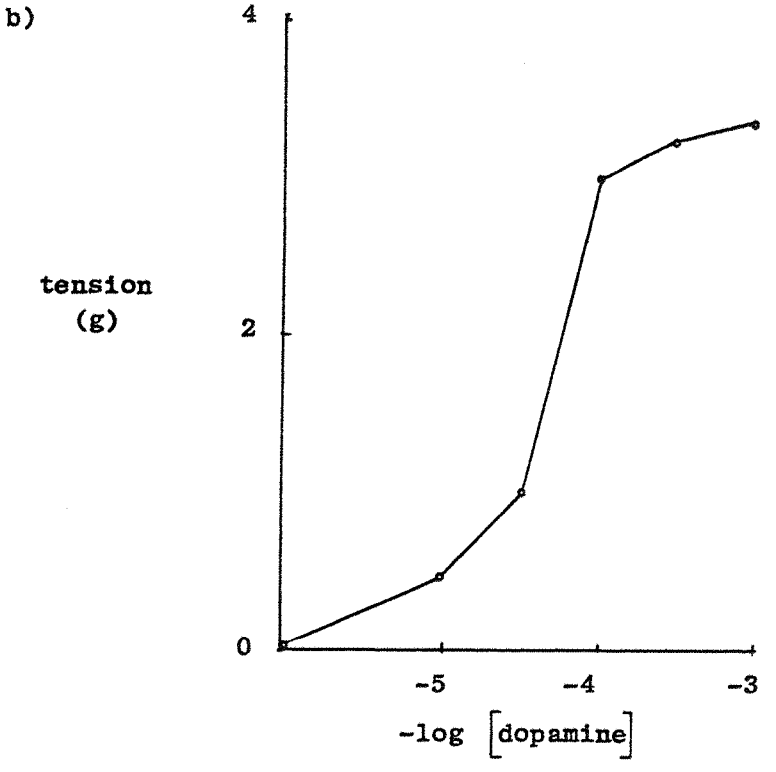


Fig. 4.9

a) The variation of basal tension with organ bath temperature for the Guinea pig atria preparation.

b) A typical dose response curve for dopamine from the Guinea pig atria preparation.

tissue. The length-tension relationship was evaluated at 4 calcium ion concentrations, 2.5, 4.0, 5.5 and 7.5mM (Fig.4.10). Increasing the calcium ion concentration potentiated the force of contraction, indicating that the contractile state of the atrial tissue was influenced by the calcium ion concentration.

4.4.6 The effect of plasma from burned and mock-burned rats on isolated guinea pig atria.

The length-tension relationship was measured at three different calcium ion concentrations (2.5mM, 4mM, 5mM), then 1ml of plasma from either a burned or a mock-burned rat was introduced into the bathing medium. The length-tension relationships for the three different calcium ion concentrations were then re-assessed in the presence of the plasma.

The length-tension relationships obtained before the addition of plasma were compared with those obtained after the addition of plasma. The percentage difference between contractile performance before and after administration of plasma from mock-burned and burned rats is shown in Fig.4.11a. The same calculations, using the measurements of maximum rate of generation of tension (dT/dt) instead of tension, gave very similar results (Fig.4.11b). When plasma from mock-burned rats was added to the bath, there was a potentiation of the force of contraction, and the greater the calcium ion concentration, the greater the potentiation. However, when plasma from burn injured rats was added to the bath, there was a deterioration in the force of contraction at each of the different calcium ion concentrations.

4.4.7 The effect of plasma taken from burned and mock-burned rats on the cardiovascular function of the anaesthetised rat.

Plasma samples were obtained fron burned and mock-burned rats as previously described. The plasma (3 ml) was then infused into the femoral vein of a pentobarbitone anaesthetised rat, while 3ml of blood was simultaneously removed from the rat via the femoral artery. The transfer of plasma was completed within 4 mins. Cardiovascular function was measured by impedance cardiography (as previously described) at the following times after infusion of the plasma: +5, +10, +15, +20 and +30mins.

The rats which had received plasma from mock-burned donors, showed small but statistically significant changes in cardiac output during the 30mins. following the

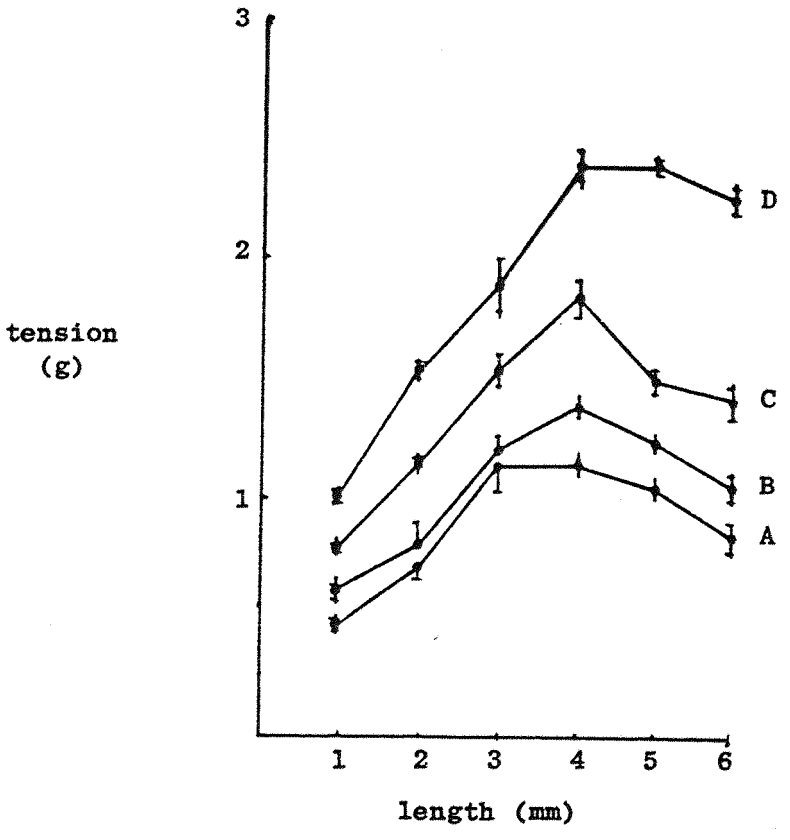


Fig. 4.10
The length vs tension relationship for the isolated guinea pig atria preparation at different concentrations of calcium ion :-

A - 2.5 mM C - 5.5 mM
B - 4.0 mM D - 7.5 mM

The values shown are mean ± SEM, where n = 6 per group.

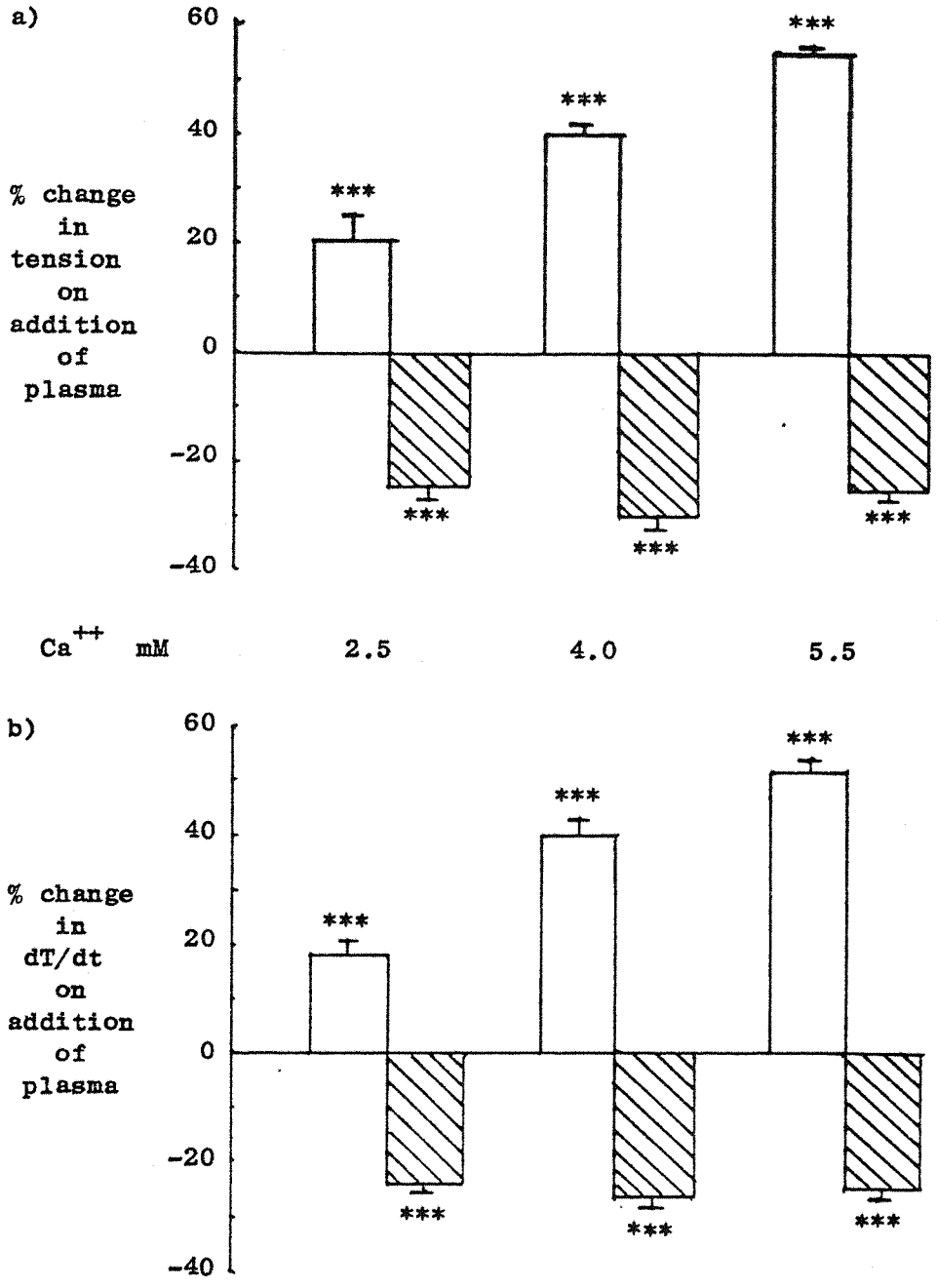


Fig. 4.11
The percentage changes in

a) tension (T)
b) dT/dt

after addition of 2ml plasma from mock-burned and burned rats to the bathing medium, in the presence of differing calcium ion concentrations. The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - $P < 0.05$ ** - $P < 0.01$ *** - $P < 0.001$

□ - received plasma from mock-burned rats
▨ - received plasma from burn injured rats

transfusion, although the cardiac output did tend to decrease. Rats which received plasma from burned donors, showed a significant decrease in cardiac output after only 5mins. (-13.7 ± 3.1%, n=6, P<.01) and this depression of cardiac output was still present after 20mins (-18.5 ± 6.3%, n=6, P<.05, Fig.4.12a).

This slight depression of cardiac output in the control rats was related to a slight depression in stroke volume, as the heart rate showed no significant change from pre-infusion values (Fig.4.12b). However the depression in cardiac output caused by the plasma from burned rats was due mainly to a significant depression of stroke volume, as the heart rate decreased initially after 5 mins, but returned to a level not significantly different from pre-infusion values.

4.4.8 Changes in blood gases and acid-base balance in anaesthetised rats after burn or mock-burn injury.

Measurements of pH, PCO_2 and PO_2 were taken at +15, +30, +60 and +90 mins after burn or mock-burn injury.

There was no significant difference in the pH of arterial blood taken from mock-burned rats (7.37 ± 0.0176, n=6) and burned rats (7.40 ± 0.0149, n=6) at 30 mins post-burn or mock-burn (Fig.4.13a).

The PaO_2 was significantly higher in the burned rats (91.9 ± 4.4 mmHg, n=6) than in the mock-burned rats (73.9 ± 4.6 mmHg, n=6, P<0.05) at 30 mins post-burn or mock-burn.

The $PaCO_2$ was significantly lower in the burned rats (34.6 ± 1.83 mmHg, n=6) than in the mock-burned rats (43.3 ± 2.31 mmHg, n=6, P<0.05) at 30 mins post-burn or mock-burn (Fig 4.13b).

There were no significant differences in plasma bicarbonate concentration or base excess, when post-burn values were compared with post-mock-burn values.

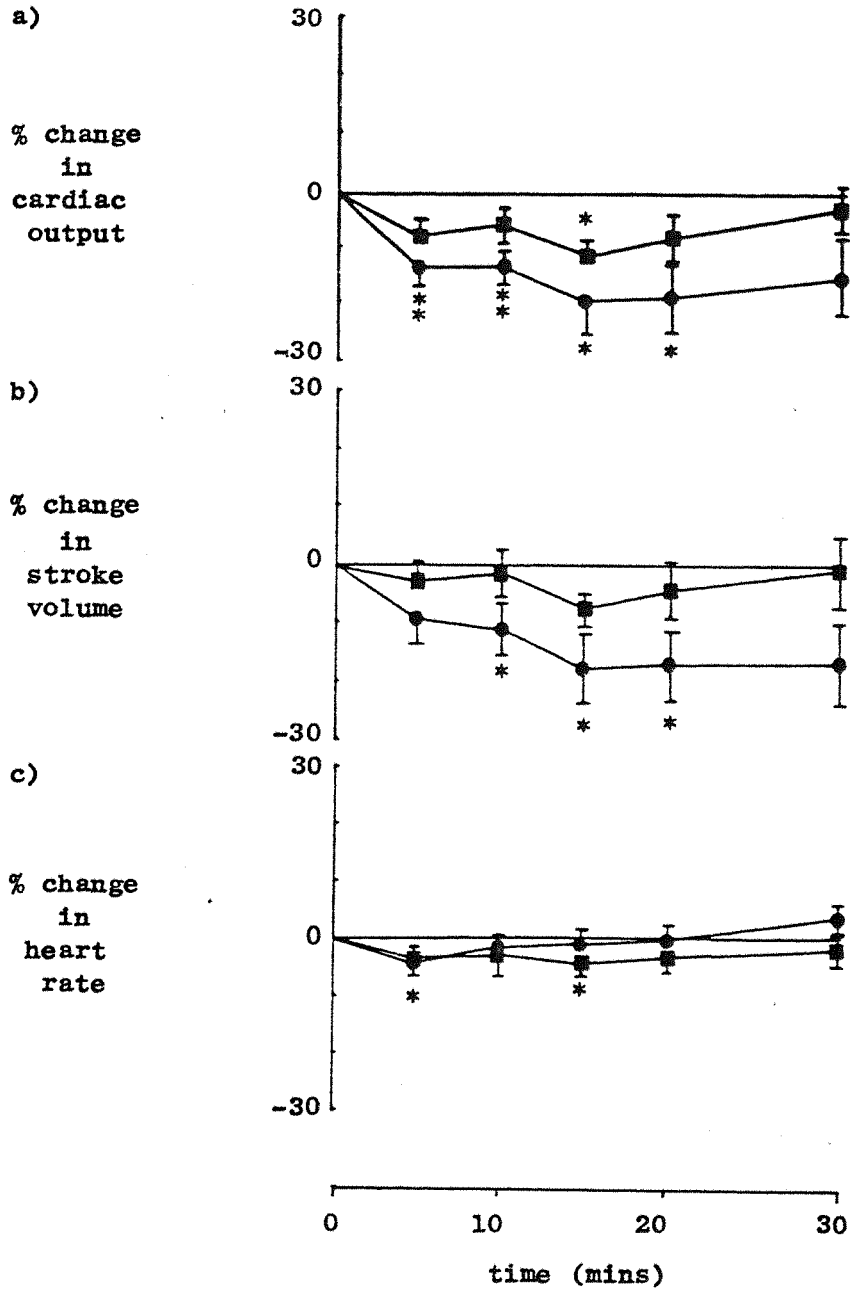


Fig. 4.12
The changes in

a) cardiac output,
b) stroke volume,
c) heart rate,

of rats which received a transfusion of plasma from mock-burned and burned donor rats. The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by paired Student's t-test.

* - $P < 0.05$ ** - $P < 0.01$ *** - $P < 0.001$

■ - received plasma from mock-burned rats.
● - received plasma from burn injured rats.

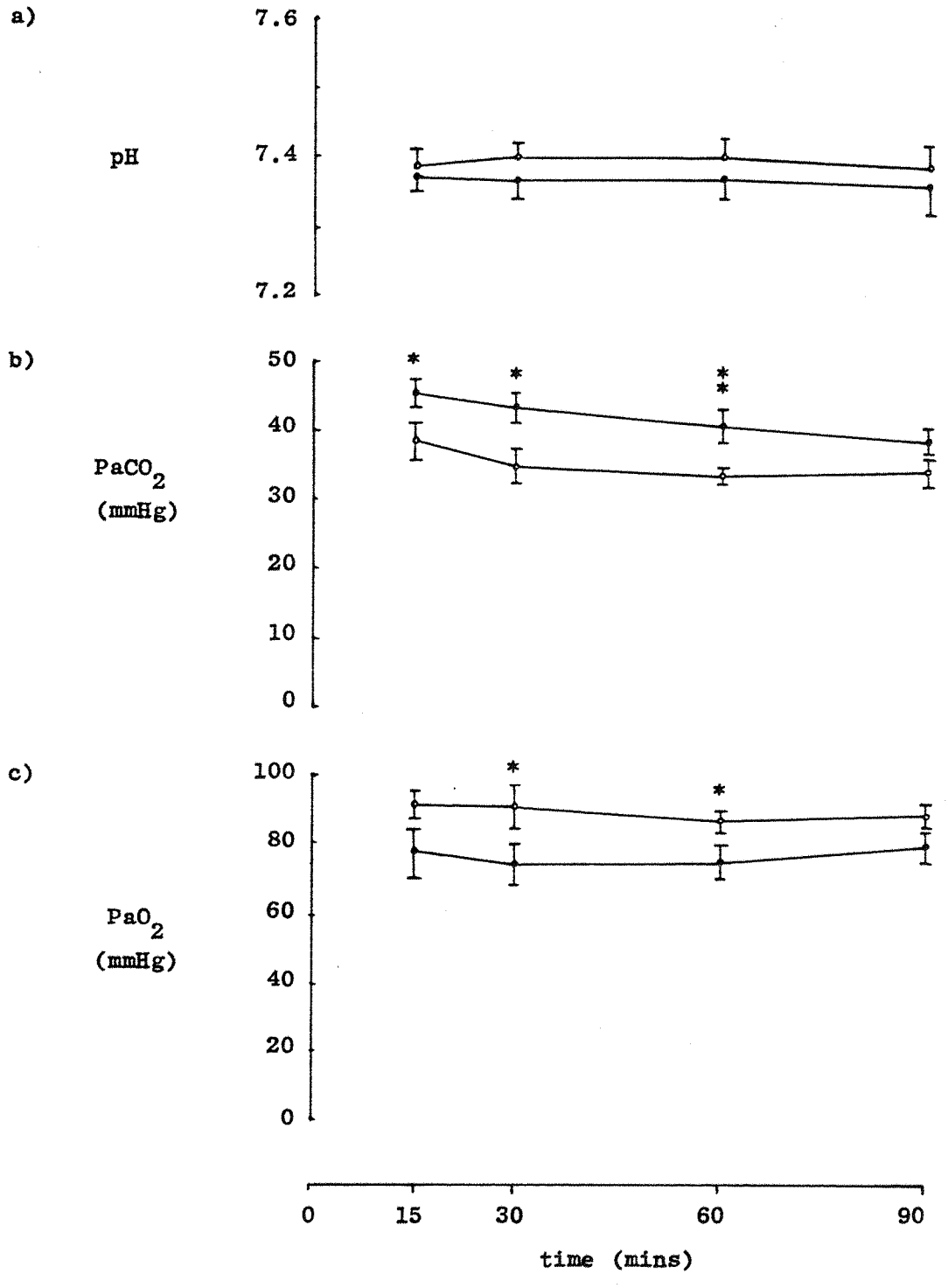


Fig. 4.13
The measurement of

a) blood pH,
b) $PaCO_2$,
c) PaO_2

after mock-burn and burn injury. The values are shown as mean ± SEM (n=6 in all groups). Results were evaluated statistically by unpaired Student's t-test.

* - P < 0.05
** - P < 0.01
*** - P < 0.001

○ - mock-burned rats
● - burn injured rats

4.5 DISCUSSION.

The studies described in chapters 2 and 3 verified that the methods employed to assess cardiac function *in vivo* in the rat were reliable, so I decided to proceed with *in vivo* investigations of cardiac function after burn injury.

The first part of this investigation was performed as two parallel experiments, that is, I alternated between the two experiments. The purpose of this was to ensure that the two experiments were carried in as near identical conditions as possible. It is then possible to infer that any cardiovascular changes observed in one experiment also occurred in the other. The first experiment was to measure the cardiac output and general cardiovascular function, before and after burn injury. The second experiment was to measure indices of cardiac contractile function before and after burn injury. The experiments were also performed using mock-burn injury, our control procedure for burn injury.

In these experiments, the stroke volume and cardiac output deteriorated within minutes of the burn injury, in a similar way to that described in chapter 1, while the blood pressure decreased initially and then stabilised at a value below the pre-burn value during the early post-burn period. These observations are consistent with studies reported earlier in this thesis and with those of Philpot (1980), who used a similar experimental model.

The majority of the indices of contractility were depressed during the first hour after burn injury. In the present study, APD and dP/dt_{max} were greatly depressed after burn injury, while ARPD and d^2P/dt^2_{max} were depressed to a lesser extent (although still significantly depressed).

In vivo measurements of ventricular contractility are difficult to interpret, even when they are obtained under conditions where preload, afterload and heart rate are controlled. In the present study, indices of ventricular contractility were measured after burn injury. As severe burn injury is known to cause progressive changes in the preload and afterload on the heart (Michie, 1963; Blalock, 1930) the results of this study must be interpreted with reference to the findings of chapter 3.

The effect of heart rate on the contractile indices can be considered by comparison of ARPD with FARPD, indices which differ only by a term proportional to heart rate. The extent of the depression in contractility

during the first hour post-burn, was lessened when corrected for heart rate, a small decrease in heart rate during the early post-burn period being responsible for this effect. The importance of this observation is dependent upon the accuracy of the findings of Moreno et al, (1976), that ARPD was related to heart rate by a square power function.

The period of maximum depression of the indices in general, was around 20 mins post-burn. This coincides with the time of greatest depression in the stroke volume of the rats in the parallel study, suggesting a relationship between these responses.

It is also important to determine whether burn injury caused significant changes in the afterload on the heart, as the work described in the previous chapter suggests that moderate increases in afterload tend to potentiate the contractile indices. Such afterload-induced potentiation of the contractile indices could mask any depression in the indices of contractility due to the direct actions of burn injury on the heart. In this study the mean arterial blood pressure (a measure of afterload) fell during the first 20 mins and then began to rise back toward pre-burn levels, indicating no overall increase in afterload. If the heart is damaged or compromised, then the arterial blood pressure may not be a good indicator of afterload (as explained in chapter 1). In this situation, the peripheral conductance becomes a better indicator of afterload. In the present experiments, however, the TPR was not raised until at least 30 mins post-burn, suggesting that the afterload was not an important causative factor in the depression of the contractile indices, which occurred prior to 30 mins post-burn. It is possible, however, that the apparent recovery of the indices after 30 mins could be due to an afterload-induced increase in contractile state.

The CVP (a measure of preload) was not measured in this study, but in a similar study using the same model of burn injury, Aggarwal, (1983) found that the CVP decreased slightly during the first 15 mins post-burn (Fig.4.14). In his study, there was no significant difference between the CVP's of burned or mock-burned rats during the first hour post-burn, however, the difference in CVP apparent in his study, could have been responsible for a 10-15% fall in the contractile indices (as assessed by a 0.4mmHg fall in CVP in the studies described in chapter 3). Unfortunately, the reduction in preload produced by haemorrhage may exaggerate the true depressor effect on the indices of contractility, as the occurrence of direct myocardial

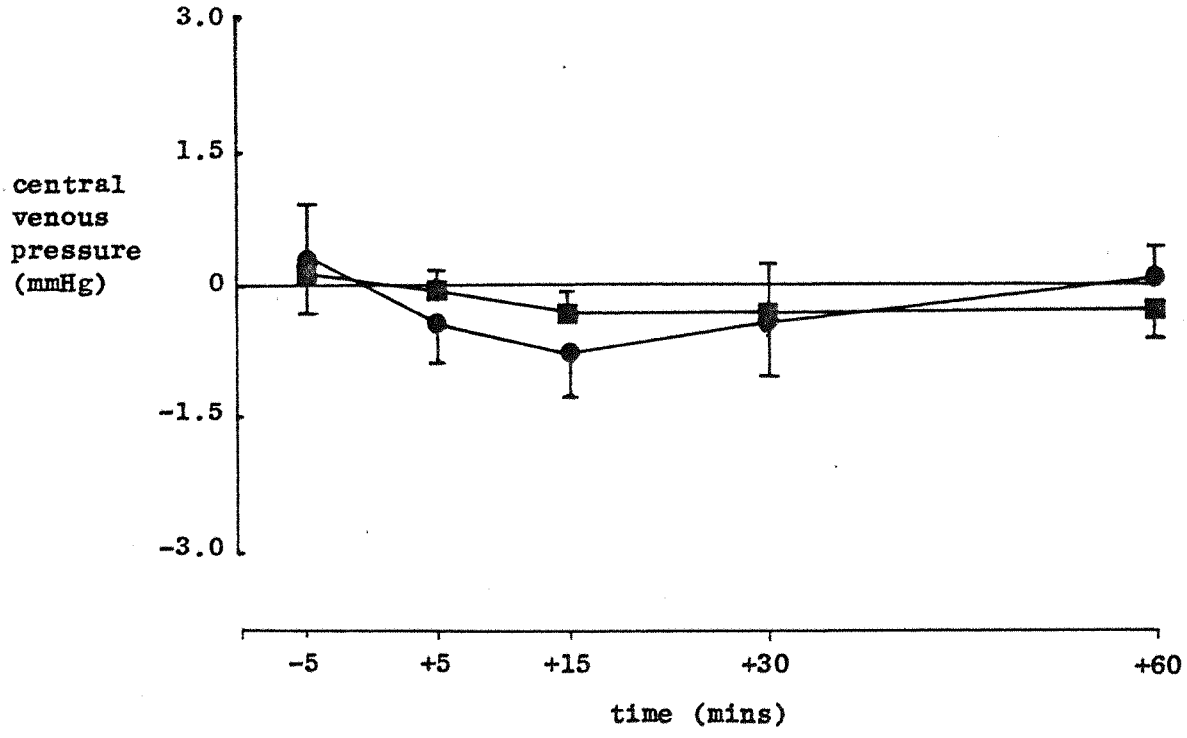


Fig. 4.14
The changes in central venous pressure occurring after mock-burn and burn injury in the anaesthetised rat. (taken from Aggarwal, 1983). Values are mean ± SEM , n=6 per group

* - P < 0.05
** - P < 0.01
*** - P < 0.001

■ - mock-burned rats
● - burn injured rats

depression has also been reported during haemorrhagic shock. However, this effect takes time to develop and it is unlikely that it could have potentiated the fall in the indices significantly. The strongest argument against the preload-induced depression of the contractile indices is the time course of the changes. Myocardial depression appeared to be most marked 20 minutes after burn injury yet CVP does not decrease sufficiently to be the cause of such a depression.

These in vivo experiments therefore suggest that there is a depression of myocardial contractility very soon after burn injury, which occurs before the changes in preload and afterload have progressed to a level where they could significantly influence the contractile indices.

Okamoto et al, (1974) and Philpot, (1981) predicted that in vivo contractile function is impaired soon after burn injury. Several other recent studies also support this finding. Kuzin et al (1983) investigated cardiac function in vivo, before, during and after burn injury to 35% of the body surface area of anaesthetised dogs. They demonstrated decreases in several indices of contractile function (dP/dt_{max}; dF/dt; V_{CE}; V_{max}), which were statistically significant by 20 minutes post-burn. In a parallel set of experiments the same workers perfused a normal isolated dog heart with blood from a burn injured dog and constructed ventricular function curves to assess contractile function. They found that ventricular function was unchanged by perfusion with blood from a burned donor, suggesting that endogenous circulating factors are not important in the depression of myocardial function, but that the nervous system of the burned animal is somehow involved in depressing myocardial function. This finding is supported by evidence that the heart is depleted of catecholamines after a burn injury (Cova & Glaviano, 1978).

The guinea pig model of 'burn shock' (Wolfe & Miller, 1976) has recently been used to investigate changes in the contractile performance of isolated hearts. Hearts excised from guinea pigs at 2, 4 and 8 hours following burn injury to 35% of body surface area showed evidence of impaired contractile function (Temples, Burns, Nance & Miller, 1984). A decline in cardiac function, which was maximal at 4 hours post-burn, was observed. Left ventricular function curves revealed a progressive downward or rightward shift, indicating a

decrease in cardiac contractility, which was accompanied by decreases in coronary flow and myocardial oxygen consumption as well as a decrease in left ventricular compliance. These investigators suggested that the changes in contractility could have been due to several causes:

i) a larger ventricular volume and decreased efficiency in the conversion of chemical energy to pressure,
ii) decreased ability to utilise oxygen,
iii) impaired oxygen delivery,
iv) a change in the coronary vasculature.

These workers thought it likely that the observed changes were caused by myocardial ischaemia. Adams et al, (1982) studied atrial muscle from guinea pigs, 16 hours after a burn to 16% of the body surface area. The systolic performance of these atrial preparations was not significantly reduced after the burn injury, but there was evidence of an abnormality during relaxation in the atrial muscle, which, according to Brutsaert,(1980), may indicate cardiac insufficiency long before systolic performance begins to fail. Myocardial dysfunction has not previously been reported after such moderate burn injuries. Adams, Baxter & Izenberg, (1984) showed that 24 hours after a burn to 47% of the body surface area, left ventricular function deteriorated significantly, as did coronary blood flow. However, coronary vasodilation failed to improve contractile performance, suggesting that ischaemia was not a major factor in the depression of ventricular performance.

The cause of this sudden depression in myocardial contractility is unknown, but there are many possible explanations. One possible cause of cardiac dysfunction is hypoxia. Hypoxia can be caused by insufficient oxygen in the blood or by a diminished coronary blood supply. The present study demonstrated that the PaO_2 increases after burn injury (due to hyperventilation, Rooke, 1984). The supply of blood to the heart was not measured in this study, but coronary vasoconstriction has been observed by several workers (Okamoto et al 1974; Rubani et al 1983; Temples et al 1984). These measurements were generally taken more than an hour post-burn, a time when coronary vasoconstriction could be a result of severe progressive circulatory shock and not necessarily its initiating factor.

Low blood pH also depresses cardiac function (Ter Keurs, 1972) but the present measurements show that the blood pH was close to normal after burn injury. The blood

concentrations of potassium, calcium and magnesium ions are unchanged in the first hour after burn injury (Aggarwal, 1983), so this would seem to rule out changes in these ions as causes of cardiac depression. A recent hypothesis has been proposed to explain the occurrence of myocardial depression after burn injury. Rubani, Szabo, Balogh, Bakos, Gergely & Kovach, (1983) observed a five-fold increase in serum nickel concentration in rats subjected to 25% body surface area burns, and ultrastructural abnormalities were observed in myocardial tissue which correlated well with abnormal subcellular localisation of nickel. Trace amounts of exogenous nickel have been demonstrated to induce coronary vaso-constriction, myocardial depression and ultrastructural changes in the myocardium (Rubani, Ligeti & Keller, 1980). The source of the endogenous nickel has not been established, but the myocardium has been proposed as a possibile site. Furthermore, elevated serum nickel levels have been observed in human burn victims (McNeely, Sunderman, Nechay & Levine, 1971). Many of these observations were made as long as 7 days post-burn, but it is interesting to note that the serum nickel concentration had increased by 230% at 3 hours post-burn and that the coronary vessels showed greatest sensitivity to nickel at this time.

An alternative explanation is that heart function is depressed by toxic sbstances, possibly released from the site of the burn and carried in the blood to the heart. Experiments in this laboratory by Banner *et al* (1980) showed that the transfer of blood from a burned donor rat to a recipient rat induced a decrease in cardiac output in the recipient rat. This property of blood from burned rats was localised to the plasma fraction (Philpot, 1980).

This chapter also describes *in vitro* experiments designed to investigate the possibility of plasma-borne factors affecting cardiac function immediately after burn injury. Plasma taken from burn injured rats caused a significant depression in the contractile performance of an isolated heart muscle preparation, when compared with the effect of plasma taken from mock-burned rats under identical conditions. This effect has been reported in several species of animal, from *in vitro* and *in vivo* cross-circulation experiments, but the most significant aspect of this study is the timing of the transfusion of plasma. The majority of previous studies of blood-borne cardiotoxic substances have used blood from patients or animals subjected to very

large burns, and which has been collected a considerable time after burn injury, at a time when burn shock is pronounced and cardiotoxic materials can appear as a consequence of shock itself. The present study is important because the plasma was collected at 5 minutes after burn injury, at the time when the cardiac output is falling rapidly. On exposure to this 'burned' plasma, the isolated atrial tissue showed a serious impairment of its ability to increase contractile state on the addition of calcium ions. This effect is highlighted by the potentiation of contractile state caused by the application of 'mock-burned' plasma. Measurement of the pH of the bathing solution revealed that the changes in contractile function could not be attributed to changes in pH. Previous experiments have shown that 5 mins post-burn, the plasma concentration of Na^+, K^+ and Ca^{++} are not significantly changed (Aggarwal, 1984). This experiment strongly suggests that a factor introduced into the plasma as a consequence of burn injury inhibits cardiac function.

The finding that the transfer of plasma causes a reduction in contractile indices agrees with the *in vivo* study and adds further evidence to support the hypothesis that there is a depression of myocardial contractility soon after burn injury, which is independent of afterload and preload and could be due to a blood-borne factor.

SUMMARY

In the present study, burn injury was produced in the laboratory rat by exposure to water at 90°C for 20 seconds. This is known to produce a full thickness burn to the skin (Philpot, 1981), while causing only a small rise in the temperature of underlying organs (Banner, 1980).

Cardiac output was measured during the early post-burn period using impedance cardiography, a method which has been validated for use in the rat and found to give reliable measurements under many experimental conditions, including burn injury (Philpot, 1981; Griffiths et al, 1981). Changes in cardiovascular function observed after burn injury were always compared with changes in cardiovascular function of rats which had received a mock-burn, the control procedure for burn injury. The following changes in cardiovascular function were observed after burn injury:

i) The cardiac output fell by 30% in the early post-burn period and remained depressed for the remainder of the 90 minute observation period (see Fig.4.1a). Similar results have been reported by Wolfe & Miller (1975) and Philpot (1981).
ii) Stroke volume showed a change parallel to that of cardiac output (see Fig.4.2b). Similar changes have been reported by Wolfe & Miller (1975) and Philpot (1981).
iii) Heart rate showed no significant change (see Fig.4.2b).
iv) Arterial blood pressure fell during the initial post-burn period, but then rose and stabilised at a level below mock-burn values (see Figs.4.1b, 4.4b).
v) Peripheral conductance rose initially but then tended to decrease to below pre-burn levels (see Fig.4.3).
vi) Haematocrit increased in value during the early post-burn period and remained significantly elevated for the duration of the observation period (see Fig.1.15).

Any change in cardiac output must be due to changes in heart rate and/or stroke volume. In these experiments, heart rate changed little after burn injury, as reported by many other workers (Baxter, 1966; Wolfe & Miller, 1975;

Philpot, 1981). The change in cardiac output must therefore be due to the fall in stroke volume. The stroke volume is determined by the following 3 factors:

i) preload on the heart,
ii) afterload on the heart,
iii) myocardial contractility.

A widely accepted explanation for the decrease in cardiac output is that a decrease in blood volume causes a decrease in the preload on the heart and sets in motion the downward spiral of circulatory shock (Davies, 1981, see Fig.1). There is, however, increasing evidence that a fall in blood volume is not the cause of the initial rapid fall in cardiac output (see section 1.2.2), so I decided to investigate the other possibilities.

Since there is no increase in mean arterial blood pressure after burn injury, there can be no increase in afterload to influence the emptying of the heart.

Philpot (1981), observed that angiotensin II may play an important role in the physiological response to burn injury. She measured plasma renin activity and found it to be increased by 400% soon after burn injury. In one of my initial experiments I used captopril, a blocker of the renin-angiotensin system, to prevent the formation of angiotensin II after burn injury. Captopril administration before burn injury modified the cardiovascular response in the following ways:

i) prevented the expected decrease in peripheral conductance (Fig. 1.6)
ii) exaggerated the fall in arterial blood pressure
iii) prevented the falls in stroke volume and cardiac output (Figs. 1.9, 1.10).

The fall in stroke volume after burn injury is normally associated with a decrease in peripheral conductance. Captopril administration prevented the expected decreases in both peripheral conductance and cardiac output, suggesting that these may be related. Other evidence also suggests that the decrease in peripheral conductance may be important in causing the decrease in cardiac output after burn injury (see section 1.2.2b).

A decrease in peripheral conductance does not usually have a great effect on the output of a normal heart, but can depress cardiac output in a failing heart (Cohn & Franciosa (1973), see section 1.2.2), even though the arterial blood pressure may be unaltered. These

observations therefore led to the hypothesis that cardiac function is depressed after burn injury, i.e cardiac contractility is depressed.

The next stage of my investigation was to assess heart function directly in the burn injured rat. Cardiac contractility has rarely been assessed in vivo in the rat, because of the problems caused by the small size of the left ventricle and the limitations of generally available pressure recording systems. The measuring system I developed, used a conventional pressure transducer with a fine bore polyethylene catheter to measure left ventricular pressure waveforms. I also developed a micro-computer system for recording the LVP, for measuring the degree of distortion introduced into the LVP measurements by resonance and damping in the measuring system and for the mathematical removal of this distortion from the LVP measurements. The resulting (or corrected) LVP waveform was then processed to yield many different indices of contractile function. Part of this work involved the development of a new technique for measuring the frequency response characteristics of physiological pressure measurement systems, which I have called the 'drop-test'.

Once a pressure measurement system with adequate frequency-response characteristics had been devised, the method was validated for use in the anaesthetised rat preparation. This section of my investigation involved observing changes in the indices of contractility in response to drug induced changes in heart function. Adrenaline and calcium ions were used to induce a positive inotropic response and Carbachol (a longer lasting analogue of acetylcholine) and verapamil (a calcium channel blocker) were used to induce a negative inotropic response. All of the indices of contractility except Vmax, changed as expected in response to these drugs (see Fig.3.6). Another experiment assessed the sensitivity of the indices to graded doses of dobutamine, a potent positive inotrope. The results showed the indices based on the second derivative of LVP (i.e. d^2P/dt^2_{max}. ARPD and FARPD) to be the most sensitive (see Fig.3.8). In an attempt to assess the preload and afterload dependence of the indices, two further experiments were performed; the afterload on the heart was increased by administration of phenylephrine (an α-adrenoreceptor agonist) and the preload on the heart was decreased (by removal of blood). These experiments showed that severe changes in afterload and preload caused relatively small changes in the contractile indices (see Figs.3.10, 3.12). The experiments described above

therefore showed that the system I had developed was capable of reliably measuring indices of cardiac contractility in the anaesthetised rat.

The final stage of my investigation was to measure these indices of contractility before and after burn injury. As the technique I had developed could not be used simultaneously with impedance cardiography, another set of experiments was performed to provide parallel data on cardiovascular function for comparison with the ventricular function data. The results from these experiments showed that there was a decrease in the contractile indices during the early post-burn period. In the first 20 minutes of the post-burn period, dP/dt_{max} and APD were reduced by 40%, d^2P/dt^2_{max} reduced by 30% and FARPD reduced by 15% (see Figs.4.5, 4.6, 4.7, 4.8). The depression in myocardial contractility corresponded with the depression of stroke volume (see Fig. 4.2b).

The indices of contractility improved 30 minutes after burn injury and returned to pre-burn levels by 60 minutes post-burn. The reason for the recovery in myocardial contractility is not known.

These results (and those described in the next paragraphs) therefore support the hypothesis that myocardial dysfunction is involved in the initial falls in stroke volume and cardiac output. The next question to be answered concerns the cause of the myocardial dysfunction.

I performed several other experiments which were designed to investigate the hypothesis that the blood of a burned animal contains a toxic factor which can induce myocardial dysfunction. A relatively small quantity of plasma taken from a rat 5 minutes after a burn injury caused deterioration of cardiovascular function in a recipient rat (see Fig.4.12). This plasma also caused considerable impairment of contractile function in an *in vitro* guinea pig atrial preparation (see Fig.4.11).

This evidence therefore strengthens the hypothesis that a cardiotoxic factor is liberated into the bloodstream by burn injury. The resulting myocardial depression and the decreasing peripheral conductance cause a decrease in the stroke volume (Fig.2).

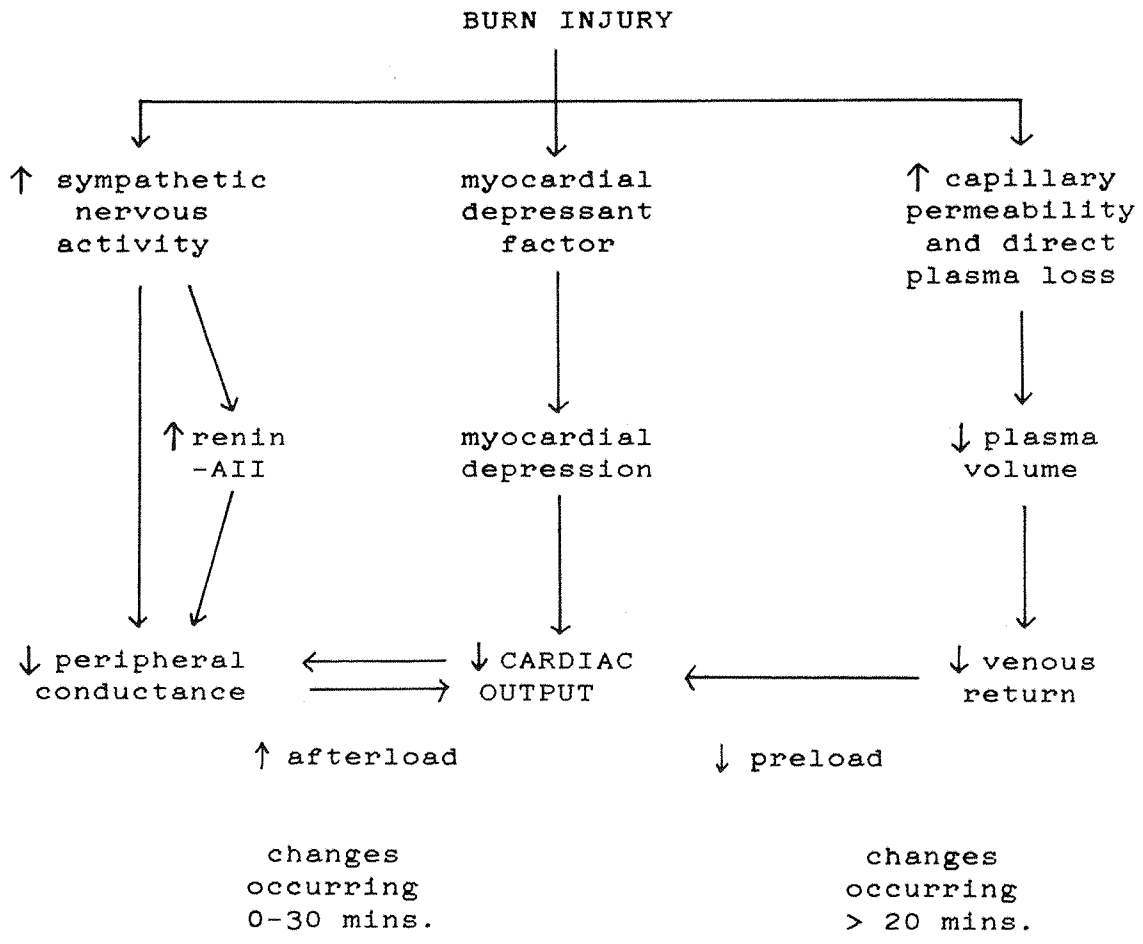


Fig.S1. A Summary of the changes occurring during the early post burn period (0-30 mins.).

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