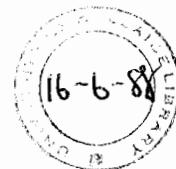


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TITLE

An isotope washout technique to study skin perfusion pressure and vascular resistance in diabetes, hypertension and peripheral vascular disease



Thesis submitted in June 1986 for the degree
of Doctor of Medicine in the University of
Adelaide

by

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The work described was performed within
the Department of Surgery of the
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SUMMARY

The rational management of ischaemic ulcers and gangrene of the lower limb is dependent upon the accurate assessment of skin blood flow. In this thesis an isotope washout technique was developed which allowed estimation of the skin perfusion pressure (SPP) and skin vascular resistance (SVR). To measure the SVR it was necessary to paralyse the vascular bed under study to eliminate non-structural factors influencing vascular diameter. An animal experiment indicated that histamine and nitroprusside were the most potent vasodilators. In individuals free of diabetes, hypertension and peripheral vascular disease (PVD) the SPP in the leg was equivalent to the systemic mean arterial pressure. In patients with PVD the SPP was reduced in proportion to the severity of the vascular disease. It is proposed that the SPP is an objective measure of PVD and is not limited by the same restraints as ankle pressures, this is, false results due to arterial rigidity.

The SVR was measured in hypertensive and diabetic patients and was found to be elevated in these groups. It is proposed that the SVR is a quantitative measure of

microvascular disease which is known to occur in these diseases.

By studying the SPP and SVR in a group of patients with ischaemic ulceration and gangrene of the lower limb it was possible to categorise the patients into three groups depending on the etiology of the ischaemia. In the first group, which are most suitable to reconstructive surgery, the ischaemia was due to atherosclerotic disease of the main arteries. This group had reduced SPP but normal SVR. In the second group, which will have little benefit from arterial surgery, the ischaemia is due to microvascular disease, as indicated by high SVR and normal SPP. The third group had a combination of large and microvessel disease.

The SPP and SVR were also useful indicators of healing of ulcers and local amputations. If the SPP $< 40\text{mmHg}$, or between $40\text{-}50\text{mmHg}$ and SVR > 1000 units, then healing was unlikely to occur and early arteriography and arterial surgery was indicated. If the SPP $> 50\text{mmHg}$ then conservative management is indicated although if the SVR is high this may not be successful.

By determining the SPP and SVR it was possible to determine the aetiology of lower limb ischaemia and to predict the likelihood of healing thus rationalising the management of these patients.

DECLARATION

I hereby declare that the work presented in this thesis is original work and was performed by myself except where otherwise stated. None of this work has been, nor will be, submitted for any other degree or diploma. I consent to this thesis being made available for loan and photocopying.

Signed,

HENRY J. DUNCAN

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PUBLICATIONS

This work is based on the following publications:

- (i) DUNCAN, H.J., FARIS, I.B., DeYOUNG, N.J.
The effectiveness of local injections of vasodilating agents to produce vasodilation in subcutaneous tissue in rabbits.
Clin. Physiol. 1985, 5:71-80.
- (ii) DUNCAN, H.J., FARIS, I.B.
Martorell's hypertensive ischemic leg ulcers are secondary to an increase in the local vascular resistance.
J. Vasc. Surg. 1982, 2:581-584.
- (iii) FARIS, I.B., DUNCAN, H.J.
Skin perfusion pressure in the prediction of healing in diabetic patients with ulcers or gangrene of the foot.
J. Vasc. Surg. 1982, 2:536-540.
- (iv) DUNCAN, H.J., FARIS, I.B.
Evaluation of an isotope washout technique to measure skin vascular resistance and skin perfusion pressure: influence of age, site and arterial surgery.
Clin. Sci. 1986, 70, 249-255.
- (v) DUNCAN, H.J., FARIS, I.B.
Skin vascular resistance and skin perfusion pressure as predictors of healing of ischemic lesions of the lower limbs : influences of diabetes mellitus, hypertension and age.
Surgery, 1986. In press.

CHAPTER 1

INTRODUCTION

The concept of a microcirculation uniting the arterial and venous sides of the circulation was introduced by William Harvey in 1628. Marcello Malpighi in 1661, with the aid of magnifying lens, reported the presence of 'tubules' through which blood flowed from arterial to venous vessels (Young, 1929). Marshall Hall (1831) was the first to attempt to differentiate between arterioles, capillaries and venules. Sucquet (1862) described another component of the microcirculation of the skin. These are the arteriovenous anastomoses, or shunts, which permit blood to pass directly from the small-sized arteries and arterioles into the venous channels, thus bypassing the capillary bed.

The function of the skin microcirculation is twofold. The capillary circulation is responsible for the supply of metabolic substrates and removal of wastes from the cells of the cutaneous tissue. This is the primary function of the

microcirculation of any organ or tissue, for impairment of capillary flow will result in a deterioration of cell function which, if severe, may lead to cell ischaemia and necrosis. The second function, the arteriovenous anastomoses, which allow large volumes of blood to flow through the skin, are important in thermoregulation.

Impaired tissue blood flow to the muscles and skin of the lower limb results in the clinical conditions of intermittent claudication, rest pain, ulceration and gangrene. Advances in surgical techniques and contrast radiography have enabled identification and treatment of atherosclerotic disease affecting the leg with the consequent improvement in mortality and morbidity.

Despite large vessel disease being well understood and, within limits, treatable the role of microvascular disease in the pathogenesis of lower limb ischaemia is uncertain. This is explained by the inability to investigate the skin microcirculation in the clinical setting.

The aim of this thesis will be to develop a technique

to study the skin microcirculation of the lower limb and to apply this technique in patients with peripheral vascular disease. This will improve our understanding of the pathogenesis of lower limb ischaemia and ultimately assist in the management of these patients.

LITERATURE REVIEW

1.1 PHYSIOLOGY

The physics of blood flowing through an in-vitro tube system was studied by Poiseuille in 1842. From his observations he noted that a number of parameters influenced the amount of serum flowing through the tubes. These factors and their effect on flow are given by the Poiseuille formula which is:

$$F = \frac{\pi \Delta P r^4}{8 \eta L}$$

where: F = blood flow

P = pressure gradient

r = radius of vessel

η = viscosity

L = length of vessel

From this formula the three main determinants of capillary blood flow are the arteriovenous pressure gradient,

viscosity of the blood and the radius of the vessels supplying the capillary network. Under normal physiological conditions the pressure gradient and blood viscosity are relatively constant. Therefore, the main determinant of capillary blood flow is the radius of the resistance vessels. Wiederhielm and Weston (1973) confirmed this hypothesis when they demonstrated that, the pressure drop, which is a function of resistance to flow, was greatest across the resistance vessels.

Control of the resistance vessels is mediated by those factors influencing the active tension developed by the smooth muscle in their walls. The smooth muscle tone of the resistance vessels is controlled by the sympathetic nervous system and by local factors. The arterioles, which have a well developed muscle layer, are richly innervated by sympathetic fibres. Cronenwett (1983), using a microsphere technique, demonstrated that the arteriovenous anastomoses in the skin are almost exclusively under control of the adrenergic nerves, whereas, the arterioles supplying the capillary bed are only partially controlled by these nerves. The resistance vessels controlling capillary flow are

probably more directly influenced by local factors, such as changes in transmural pressure or changes in concentration of local metabolites and metabolic by-products (Folkow et al, 1971).

The ability of blood vessels to respond directly to changes in the local environment is known as autoregulation. The exact mechanisms of autoregulation are not known, but current theories involve myogenic and metabolic mechanisms. The myogenic properties of vascular smooth muscles were first described by Bayliss (1902) and have recently been reviewed by Johnson (1981). The myogenic theory proposes that increased transmural pressure excites the smooth muscle cell causing it to contract, and conversely, a decrease in pressure results in relaxation. Bayliss (1902) reasoned that this reaction maintained a constant blood flow to the tissues despite variations in the level of the arterial pressure.

The metabolic theory of autoregulation was first suggested by Born (1956) who noticed a direct relationship between the high energy phosphate content of smooth muscle

and its tension. Many substances have now been shown to affect local vascular smooth muscle tension. These include oxygen (Carrier, Walker and Guyton, 1964), prostaglandins (Pittman, 1979), adenosine (Belloni, Phair and Sparks, 1979) and K^+ (Murray and Sparks, 1978). Although other metabolic factors have been shown to influence smooth muscle tone the mechanism of action of these substances is not known. Despite this there is little doubt that local factors, myogenic and metabolic, play a large role in the regulation of capillary blood flow.

The presence of autoregulation in human cutaneous tissue has been demonstrated by Henriksen et al (1973) who measured cutaneous tissue blood flow, by means of an isotope washout technique, at perfusion pressures varied by limb elevation. They found that with a reduction in perfusion pressure of up to 30 mmHg the blood flow remained constant. This is one of the few studies of skin autoregulation although the phenomenon has been identified in the brain (Lassen, 1964) and kidneys (Haynes et al, 1953).

1.2 PATHOPHYSIOLOGY

From the Poiseuille formula it can be deduced that the capillary circulation can be impaired by:

- (i) decreasing the perfusion pressure (P),
- (ii) increasing blood viscosity (η) or
- (iii) decreasing the radius of the resistance vessels (r).

1.2.1 Reduction in perfusion pressure

The perfusion pressure is the difference between the arterial and venous pressures. The most common cause of a reduced perfusion pressure is a decreased arterial pressure secondary to atherosclerotic disease of the main arteries.

Non-invasive indirect measurement of distal blood pressure has become a very useful method of assessing the severity and location of arterial disease (Yao, 1970). These non-invasive methods involve the application of an arterial occlusion cuff at the level being studied and the placing of a sensing device which will detect changes in blood flow or volume distal to the cuff. Various devices

are used, the most common being Doppler ultrasound (Yao, 1970; Fitzgerald and Carr, 1977), strain-gauge plethysmography (Bell et al, 1972) or photoplethysmography (Bone and Pomajzl, 1981).

Ankle systolic pressure determined by these non-invasive means is slightly higher than the arm pressure in normal individuals. This is explained by an exaggeration of the systolic peak due to increased rigidity of the distal arterial tree (Birnstingl, 1973). A pressure index is obtained by comparing the systolic pressure in the leg with that in the arm. This pressure index is useful in grading the degree of ischaemia, being lower in patients with more severe ischaemia (Yao, 1970). One of the limitations with ankle pressure estimation is the presence of calcified arteries which reduces their compressibility (Hauser et al, 1984). This is a particular problem in diabetic patients who develop linear calcification of the media known as Monckeberg's sclerosis (Ferrier, 1964; Neubauer, 1971).

The consequence of a reduction of the arterial pressure in the leg, secondary to atherosclerotic disease of the

vessels of the abdomen and leg, is impairment of capillary blood flow to the tissues of the leg. As the severity of the disease increases there is insufficient blood flow to the calf muscles during periods of increased demand as occurs in exercise. This results in the intermittent claudication. It is relieved by rest and reproduced by similar magnitude of exercise (McCombs, 1979).

In patients with mild to moderate intermittent claudication resting calf blood flow is not significantly decreased (Hillstead, 1963; Lindbjerg, 1965; Lassen and Holstein, 1974). However, direct measurements of the local oxygen tension at rest in the skeletal muscle of patients with intermittent claudication has shown a marked reduction in the oxygen tension compared to normal (Ehrly and Schroeder, 1977; Hans et al, 1977). These findings suggest that a number of compensatory mechanisms exist in patients with claudication. The reduction in distal pressure but the maintenance of normal flow suggests that the resistance to flow in the vascular bed is decreased. That is, the resistance vessels have dilated. This is most probably due to local factors which were discussed above. The decreased

oxygen tension in the resting muscles suggest that the muscle is more efficient at extracting oxygen from the haemoglobin. This probably results from a shift to the left of the oxygen dissociation curve secondary to alterations in the CO₂ content and pH of the muscle (West, 1979). The compensatory mechanisms of vasodilation and increased oxygen extraction result in maintenance of tissue oxygen supply despite a reduction in distal blood pressure.

Muscle blood flow increases during exercise because the action of the local metabolites results in relaxation of the resistance vessels. This fall in resistance results in increased flow provided the inflow vessels are patent. In patients with intermittent claudication the inflow obstruction means that flow cannot increase sufficiently and thus ischaemia results.

As the severity of the arterial disease increases

claudication occurs with mild or minimal exertion. The patient may also complain of burning pain in the foot which occurs in the early hours of the morning. The mechanism of this rest pain is still undecided. Foot blood flow at rest evaluated by Xe-133 washout (Ahlstrom and Westling, 1971; Eickhoff, 1980) and venous occlusion plethysmography (Mune, 1967; Cuyper and Steels, 1981) was no different in subjects with normal circulation and in patients with varying severity of arterial disease. Steer (1980), also using venous occlusion plethysmography, demonstrated that when patients were subjected to reactive hyperaemia it was possible to differentiate patients with rest pain from intermittent claudication. This is a similar finding to muscle blood flow in patients with claudication and probably has the same explanation. This, however, does not explain the pathophysiology of rest pain.

Jelnes and Tonnesen (1984) used an isotope washout technique, which allowed a continuous 24 hour recording of the subcutaneous blood flow on the dorsum of the foot, to study patients with rest pain. They found that during the day hours the blood flow was similar in subjects free of

arterial disease and in patients with claudication. However, during sleep the blood flow nearly doubled in normal individuals, was constant in claudicants and was decreased by approximately 50% in the patients with rest pain. Bevan et al (1969) and Littler et al (1975) noted that mean arterial blood pressure falls by 20-30% during sleep. In normal individuals loss of sympathetic tone and autoregulation could explain the maintenance of foot blood flow at night despite the fall in blood pressure. However, in patients with rest pain the skin blood vessels are already dilated and are not able to further compensate when the blood pressure falls at night, and thus foot blood flow is further reduced (Jelnes and Tonnesen, 1984). This study has provided a possible mechanism for rest pain.

If the atherosclerotic arterial disease is very severe skin necrosis occurs with ischaemic ulceration or gangrene. Fagrell (1973) used vital capillary microscopy to study the capillary circulation in patients with severe arterial disease. This study demonstrated that even if the arterial circulation is severely impaired, ischaemic skin necrosis may not develop if the microcirculation is preserved.

However, if only a few capillaries were functional then necrosis was imminent. In some patients a digital arterial circulation which was compatible with normal function was associated with necrosis of the digit (Conrad, 1968; Fagrell, 1975; Gundersen, 1972). In these patients direct microscopic studies demonstrated marked destruction of nutritional capillaries (Fagrell, 1973). A possible explanation of these findings is that the blood was passing through the arteriovenous anastomoses, which are numerous in the digits, and not the capillary circulation. Therefore the factor which appears to precipitate tissue necrosis appears to be not only severe arterial disease but also the added insult of an occlusion, most likely microthrombi secondary to the sluggish flow, of small arterioles. In adjacent areas microthrombi may not occur and the tissue remains viable thus explaining areas of demarcation which are seen with digital gangrene.

Atherosclerotic disease results in a reduction of the arterial pressure distal to the diseased segment. With a reduction in the pressure the microvessels dilate, through the process of autoregulation, to maintain blood flow. With

increasing severity of arterial disease the compensation becomes incomplete and symptoms of ischaemia appear at times of increased oxygen demand (intermittent claudication). With very severe disease the compensation is inadequate even at time of rest (rest pain) and if microthrombi occurs and capillary flow is abolished then necrosis of the affected area occurs.

1.2.2 Increase in Viscosity and Coagulability

The study of flow properties of the blood is known as haemorrheology. In the last decade there have been many publications commenting on alteration in blood viscosity in ischaemic vascular disease. One of the reasons for this interest in haemorrheology is that by pharmacological manipulation viscosity may be reduced thus improving capillary blood flow, thereby reducing tissue ischaemia.

Many factors contribute to blood viscosity. These include plasma proteins, red blood cells, white blood cells, platelets and alterations in hydration. Brown and Griffin (1930) reported that severe peripheral vascular

complications were associated with polycythaemia vera in the absence of atherosclerotic arterial disease. Edwards and Cooley (1970) showed that 16 out of 26 patients with polycythaemia presented with vascular complications. Merrill et al (1963) noted that the problems in polycythaemia related to the increased blood viscosity secondary to increased concentration of red blood cells. This resulted in blood stasis and formation of microthrombi. Treatment of these patients involves the reduction of blood volume and red cell mass by venesection (Cranley et al, 1963) or by cytotoxic therapy (Fagrell and Mellstedt, 1978).

Primary thrombocythaemia is a recognised condition which frequently presents with haemorrhagic manifestations (Gunz, 1960; Silverstein, 1968; Lewis et al, 1972). However, vascular thrombosis and microvascular occlusive disease have also been reported (Frick, 1969; Preston et al, 1974). Singh and Wetherley-Mein (1977) studied 27 patients with primary thrombocythaemia, nine of whom presented with microvascular disease. Busulfan was effective in all patients. They emphasised that in these patients it is important to recognise that the vascular complications may

be secondary to treatable medical conditions. Failure to realise this may lead to unnecessary amputations.

Recently, with the improved technology enabling more refined measurements, the emphasis has shifted from these gross changes in blood viscosity to more subtle changes which can be seen in peripheral vascular disease, diabetes mellitus and Raynaud's disease. These 'microcirculatory disorders' which are secondary to these other conditions may result in further reduction in tissue oxygenation and, therefore, exacerbate the symptoms of tissue ischaemia.

In the microcirculation there are certain physiological functions which must be maintained for appropriate exchange of oxygen and carbon dioxide between tissue and the blood. Red blood cell behaviour, vessels wall integrity and plasma proteins all influence the movement of red blood cells along capillaries. Merrill (1969) states that, at normal haematocrit, the blood viscosity is high at low shear rates because of fibrinogen-dependent rouleaux formation. Therefore, abnormal fibrinogen levels may increase blood viscosity. Red blood cell deformability is another

important determinant of blood viscosity in the microcirculation (Weed, 1970). Red blood cell deformability is the property which allows cells with a diameter of 8 micrometre to pass through capillaries with diameter as low as 2-3 micrometre. Red cell deformability is a function of osmolarity, pH, oxygen tension, membrane condition and haemoglobin concentration (Murphy, 1967; Schmid et al, 1969; Dintenfass, 1962).

Dormandy et al (1973) studied the rheological and biochemical findings in 126 patients with intermittent claudication. They demonstrated an increase in blood viscosity at low and high shear rates in claudicants compared to non-claudicants. There was a significant correlation between the severity of disease and the increase in viscosity. Patients with claudication also had a higher fibrinogen concentration and this correlated with the viscosity. In a more recent study (Wilhelmsen et al, 1984) it was demonstrated that elevated fibrinogen was a significant risk factor in the development of stroke or myocardial infarction. Ehrly and Kohler (1976) were the first to demonstrate that in patients with arterial disease

there was a reduction in red blood cell deformability. This finding has been confirmed by Reid et al (1976) who studied 44 patients with intermittent claudication or rest pain. They also noted that with increasing severity of arterial disease there was greater reduction in red cell deformability. Local changes in ischaemic tissues such as low pH, accumulation of metabolites, a low O₂ and a raised CO₂ all increase red cell rigidity (Murphy, 1967; Dintenfass, 1962) and these factors probably explain the reduced red cell deformability seen in leg ischaemia (Farconi et al, 1979).

Dormandy (1983) on the basis of these findings proposed a concept of a 'vicious cycle' when tissue ischaemia develops. With increasing severity of atherosclerotic disease the tissues become ischaemic. Associated with the tissue ischaemia are changes to blood viscosity, plasma fibrinogen and red cell deformity. These changes further reduce capillary flow thus exacerbating the ischaemia.

An understanding of haemorrheology and its abnormalities in arterial disease has stimulated an interest

in the manipulation of the rheology to improve tissue ischaemia. Messmer (1978) and Bercut and Andrews (1979) demonstrated that tissue oxygenation and blood flow were increased by haemodilution. Drugs which improve red blood cell deformability, in particular pentoxifylline have been shown to improve, subjectively and objectively, features of peripheral vascular disease in controlled clinical trials (Ehrly, 1975; Schubotz, 1977; Bollinger and Frei, 1977; Hans et al, 1977; Porter and Baur, 1982).

Blood flow properties, or haemorrheology, are altered in arterial disease. This creates a vicious cycle where the arterial disease results in tissue ischaemia which then changes blood flow properties, and thus worsening the ischaemia. An understanding of these abnormalities has stimulated study into the pharmacological manipulation of the blood to improve its viscosity. Although the results are not conclusive it appears that these avenues of therapy may provide the clinician with an effective medical treatment for patients with peripheral arterial disease.

1.2.3 Decrease in Radius

A decrease in the radius of the resistance vessels, that is the arterioles and precapillary sphincters, will reduce capillary blood flow. Because the blood flow varies with a power function of the radius, small changes to the radius will have large effects on blood flow. Diabetes mellitus and hypertension are diseases which are associated with structural changes to the resistance vessels and, therefore, may result in impaired capillary blood flow. These two conditions will be discussed in detail.

Diabetes mellitus

Patients with diabetes mellitus are more prone to develop atherosclerotic disease of the legs than nondiabetics (Robertson and Strong, 1968; Kannel and McGee, 1928; Ganda, 1980). An interesting aspect of diabetic atherosclerotic disease is its predilection for the distal arteries, especially for the medium and small sized arteries below the knee (Strandness, Priest and Gibbons, 1964; Ferrier, 1967; Faris, 1975). Apart from atherosclerotic

arterial disease diabetics also have an increased incidence of disease of the microvessels. This became apparent after the introduction of insulin which prolonged the life of young diabetics. Kimmelstiel and Wilson (1936) described changes to the microvessels in the kidney glomerulus leading to glomerulosclerosis, and Ballantyne and Loewenstein (1943) described the presence of capillary microaneurysms in diabetic retinopathy.

Since these original reports the presence of disease of the microcirculation in other tissues had been sought. This discussion will be confined to diabetic disease of the skin. Initial studies concentrated on the functional aspects of skin blood flow in diabetics. Starr (1930) demonstrated decreased cutaneous response to histamine in the legs of diabetic patients without evidence of atherosclerotic arterial disease. Similar responses were found by Megibow et al (1953) who produced vasodilatation by sublingual nitroglycerine and by Barany (1955) who used reflex heating. These workers concluded that the abnormal responses seen to cutaneous vasodilatation in the absence of arterial disease were due to abnormalities of the microvessels.

The first detailed pathological study examining the microcirculation in diabetes was by Goldenberg et al (1959). In this classic study they examined 152 amputation specimens, varying from midhigh to digital amputations, without knowledge of clinical history. On analysis they had 92 diabetic specimens and 60 nondiabetics. There was an increased incidence of microvessel disease in the diabetic specimens which was characterised by arteriolar endothelial proliferation and the deposition of periodic acid Schiff (PAS) positive material in the basement membrane. Moore and Frew (1965) confirmed these findings and Stary (1966) and Williamson and Kilo (1977) reviewed the literature supporting the presence of diabetic microvascular changes in the skin, although LoGerfo and Coffman (1984) disputed some of their conclusions.

The functional consequences of these structural changes are uncertain. Starr (1930), Megibow et al (1953) and Barany (1955) all demonstrated an abnormal response to stimuli which precipitated cutaneous vasodilatation. They found that the response was abnormal even in the absence of

atherosclerotic arterial disease and they took this as evidence of altered function of the microcirculation. However, Moore and Frew (1965) suggested that autonomic neuropathy would produce similar responses and concluded that these findings were not specific for structural changes to the microcirculation. In a more recent study Greeson et al (1975) ^m ~~ex~~ ^{am} ~~ained~~ the cutaneous vascular responses in diabetes. They measured blood flow by capacitance plethysmography and Xe-133 washout in response to body heating and topical rubefacients (tetrahydrofurfuryl ester of nicotinic acid). The resting skin blood flow was lower in the diabetic group which also had a decreased vasodilator response. Again these authors could not distinguish between autonomic neuropathy and microvessel disease.

Autonomic neuropathy can interfere with blood flow apart from producing an abnormal vasodilator response. By using Doppler ultrasound Edmonds, Roberts and Watkins (1982) demonstrated abnormal waveform patterns in the foot arteries suggesting decreased peripheral resistance and suggested that this is due to opening of the arteriovenous shunts in the foot. This conclusion is supported by Ward et al (1983)

and Watkins (1983) who noted abnormal venous distension and high venous oxygen tension on the dorsum of the foot suggesting shunting of blood away from the capillary circulation.

Munck et al (1966) studied skeletal muscle blood flow by Xe-133 washout in diabetic patients with and without neuropathy. No patients had evidence of atherosclerotic arterial disease. Muscle blood flow was normal in both groups of diabetic subjects at rest. However, reactive hyperaemia was more rapid in diabetic subjects with neuropathy than those without, the latter being similar to the normal controls. This is in contrast to Christensen (1968) who demonstrated decreased peak muscular flow in diabetic patients and he suggested that this was secondary to medial calcification which was common in the diabetic group. Verhaegen (1976) using venous occlusion plethysmography confirmed Christensen's (1968) work but suggested that microangiopathy or neuropathy could be responsible for these findings. Neubauer (1978) used a combination of feet down tilt and ischaemic exercise to assess vascular function and found that in diabetic patients

there was a decreased ability of the vessel to distend with increasing transmural pressure induced by the tilting. He concluded that this was due to stiffening of the vessel walls in diabetic patients. However, he could not distinguish between small and large vessels.

More recently cutaneous vascular reactivity in diabetic patients had been assessed by transcutaneous oxygen estimations. Ewald, Turemo and Rooth (1981) studied a group of young diabetic patients. Reactive hyperaemia of the forearm was produced by four minutes of ischaemia. They found a smaller increase in cutaneous oxygen in the diabetic group compared to controls. They concluded that these abnormal reactions were due to microvascular disorders, but they have ignored the possibility of increased rigidity of the large vessels. Railton et al (1983) also used transcutaneous oxygen tension to measure vascular responses. In their study they heated the probe to 45°C to induce reactive hyperaemia and found similar results to the previous study. However, they suggested that the abnormal response could be due to either microangiopathy, neuropathy or large vessel disease.

Some studies using an isotope washout technique have been performed in an attempt to specifically study the microcirculation in diabetes. Faris et al (1982) measured distensibility of skeletal muscle vessels in response to tilting after ischaemic exercise or local injections of papaverine to paralyse the vascular bed. They found reduced distensibility in diabetic patients under both experimental conditions. They concluded that as the papaverine did not alter large artery pressure the response was due to changes in the microcirculation. However, they overlook the possibility that lowering leg would also distend the large and medium sized arteries and that if these are more rigid in diabetic patients then similar responses would be seen and, therefore, their conclusions may not be valid. Faris and Lassen (1982), in another study, have developed a method which gave a quantitative estimation of skin vascular resistance under conditions of maximal dilatation and, by using this method, have shown that in diabetic patients there is an increase in the resistance. They suggest that this may be due to diabetic microangiopathy as this test is not influenced by neuropathy or atherosclerotic disease.

Despite the fact that skin microangiopathy has been proved histologically and suggested by numerous physiological studies its clinical significance has not been determined. This is largely due to the lack of a suitable clinical test which is specific for microangiopathy. Strandness, Priest and Gibbons (1964) who carefully studied histologically the lower extremities of diabetic patients who had gangrene and ulceration found that in all limbs there was either occlusive arterial disease or advanced sensory neuropathy. Nielsen (1973) could demonstrate skin microangiopathy in only 4 out of 15 patients with foot lesions and all of these patients had large artery disease. These workers, therefore, concluded that microangiopathy was of little clinical importance in the development of lower limb ischaemia.

Patients with diabetes mellitus have a microangiopathy of the skin which has been proved histologically. Numerous physiological studies have demonstrated abnormal vascular response but none of these are specific for microangiopathy. The clinical significance of this microangiopathy is not

known.

Hypertension

Johnson (1868) described the presence of medial hypertrophy of the arterioles in kidneys of patients with chronic hypertension. Gull and Sutton (1872) believed that the arteriolar lesions were degenerative and not hypertrophic. Evans (1921) observed intimal proliferation and hyalinization as well as medial hypertrophy in many organs of hypertensive individuals. In the definitive study of Moritz and Oldt in 1937 the histological changes in the microcirculation of hypertensive individuals were characterised. The histological features noted were intimal hyalinisation, medial hypertrophy and degeneration, and endothelial hyperplasia. They concluded that the medial hypertrophy was an early response to the increased arterial pressure while the hyalinisation and endothelial hyperplasia were late and more permanent changes.

Following these studies most research was directed at the aetiology of these structural changes and their role in

the pathogenesis of essential hypertension. The majority of this work is outside the scope of this discussion. However, of interest is the finding that these structural changes result in narrowing of the resistance vessels and this increases the peripheral resistance (Folkow, Grimsby and Thulesius, 1958; Folkow, 1970; Silvertsson, 1970). The main interest in this finding has been the role of this elevated resistance in the pathogenesis of hypertension, and very little attention has been paid to the effect of this on capillary blood flow in the distal tissues.

Conway (1963) who measured forearm blood flow by venous occlusion plethysmography found that resting blood flow was similar in normotensive and untreated hypertensive patients. This implied that there was an elevation of forearm vascular resistance in the hypertensive patients. Under conditions of maximum vasodilatation, produced by reactive hyperaemia, the resistance was still elevated. The blood flow during reactive hyperaemia was significantly higher in normotensive than hypertensive patients indicating that this elevated resistance reduced maximum blood flow. Amery et al (1969) using Xe-133 washout to measure muscle blood flow found

similar results to the previous study. Zweifler and Nicholls (1982) using pneumoplethysmography measured the finger pulse volume in hypertensive patients and demonstrated a reduction in volume when compared with normotensive subjects, thus demonstrating that the hypertensive changes affected digital blood flow.

Hartling et al (1978) using Xe-133 washout demonstrated that muscle blood flow did not increase to the same extent when subatmospheric pressure was applied to the leg in hypertensive patients as it did in normals providing further evidence that the altered structure of these vessels affected their function. However, it is possible that rigidity of the larger arteries may have produced these results and, therefore, this work is not conclusive. Henriksen et al (1981) studied the subcutaneous vessels by Xe-133 washout and found that when vessels were paralysed by local injections of papaverine the minimum vascular resistance was elevated in hypertensive patients. The distensibility of the vessels was also reduced in this group. After 6-18 months of antihypertensive treatment the vascular resistance had returned to normal but the

distensibility was still reduced. They concluded that there were two components to the structural changes in hypertension, one being reversible with lowering of the blood pressure, the other irreversible. This would support the findings of Moritz and Oldt (1937) who noted that the medial hypertrophy was secondary to the elevated blood pressure while the endothelial changes were chronic and more permanent changes.

The only studies which have suggested that hypertensive microangiopathy may play a role in the pathogenesis of skin ischaemia are those concerned with a clinical entity known as hypertensive ischaemic leg ulcers of Martorell. These are painful ischaemic leg ulcers on the lower calf in patients without arterial and venous disease. Histology demonstrated hypertensive changes to the arterioles and it was proposed that it was these changes which caused the ischaemia (Martorell, 1945; Hines and Farber, 1946; Martorell, 1978). Apart from these studies there is little evidence that hypertensive microangiopathy plays any role in the pathogenesis of skin ischaemia.

Hypertension, therefore, is associated with well defined structural abnormalities of the microcirculation. Most attention has been focused on the importance of these abnormalities in the pathogenesis of hypertension although there is some evidence to suggest that they do interfere with peripheral blood flow. This has been supported by finding of leg ulcers which are secondary to hypertensive arteriolar disease.

1.3 ASSESSMENT OF SKIN BLOOD FLOW

The assessment of skin blood supply is essential in the management of a patient with peripheral vascular disease. An ideal test of skin blood flow should be harmless to the patient and skin, accurate, reproducible, rapid and inexpensive (Creech and Miller, 1975). There have been many methods devised for assessing skin blood flow. These may be categorised into four groups.

1.3.1 Clinical Tests

These involve observation of skin colour and temperature, capillary refilling time and, intraoperatively, bleeding characteristics. These methods are often inadequate as they are subjective even for experienced observers. However, an adequate clinical assessment is critical as this will determine which patients require further, objective assessment.

1.3.2 Chemical Methods

Intravenous injections of chemical agents which diffuse out of the capillaries into the interstitial fluid where they can be detected have been used to assess capillary blood flow. The most commonly used agent has been fluorescein. Failure of an area of skin to stain implies inadequate perfusion (Lange and Boyd, 1942). This method has been used to assess the viability of skin flaps used in reconstructive surgery (McCraw et al, 1977), but its use is limited as staining takes up to 24 hours to clear.

1.3.3 Instrumental Methods

There have been a number of instruments developed to assess skin blood flow. The most widely used are temperature estimation, transcutaneous oxygen measurement, photoplethysmography, Doppler ultrasound and laser Doppler.

Skin temperature measurement is a simple procedure (Stoll and Hardy, 1949) but there are associated problems. The temperature of the circulating blood is a critical

factor (Woodcock, 1975) as in the metabolic rate of underlying muscles (Biller, 1972). Large regional variations in skin temperature under normal circumstances have also been reported (Eddy and Taylor, 1931). Baptista (1970) used the skin temperature estimation to study peripheral vascular disease but it has limited application because it measures total and not capillary, or nutritional, blood flow (Challoner, 1976) and is affected by changing ambient temperatures (Leonard et al, 1982). The measurement of skin temperature, therefore, adds little to the clinical evaluation of the patient.

Photoelectric plethysmography was introduced by Hertzman (1938) to assess skin blood supply. This method involves a light source directed at the skin. Some of the light is absorbed but some is reflected by the red blood cells and can be detected by a photoconductive cell. The amount of light absorbed depends on the blood content of the tissue and this varies with the pulse. The photocell output may then be amplified to produce a pulse wave. The fundamental process on which the technique rests is still poorly understood (Nijboer et al, 1981). The term

plethysmography is inaccurate because volume changes are not measured. Despite this the title has received general acceptance. Photoplethysmography (PPG) has been used to assess the results of sympathectomy (Hertzman and Dillon, 1940; Metz, 1955; Simonson, 1956). Eldrip-Jorgensen et al (1966) used PPG to assess effectiveness of vascular surgery. Ramsay and Challoner (1976) demonstrated high skin blood flow using PPG despite virtual capillary stasis indicating that PPG measures the total skin vascular network including blood shunted through arteriovenous anastomoses. That is, it measures total skin blood flow, not only nutrient flow. The other main disadvantage is its inability to give quantitative measurements in absolute units. However, PPG when used as a pulse indicator can be used to measure digital and segmental blood pressure.

Doppler shift flowmetry has become the most widely used noninvasive method of blood flow detection. It is based on the phenomenon that when a sound source moves relative to an observer a frequency change can be noted consisting of an apparent shift to a higher frequency as the source approaches and to a lower frequency as it moves away. This

principle has been applied to biological systems using red blood cells as the moving objects (Franklin et al, 1961). This method is used to measure blood flow and pressure in large and medium sized arteries (Yao, 1970; Yao et al, 1984) but is not suitable for capillary flow measurement. Recently, a laser Doppler velocimeter has become available. This device uses a fiberoptic light guide to transmit laser light to the skin and carry back scattered reflected light to photo detectors (Stern, 1975). This method has been compared with Xe-133 washout in cutaneous and has been found to give a good correlation (Holloway and Watkins, 1977). However, this method has a number of disadvantages. It is affected by skin pigmentation, can not be absolutely calibrated for skin blood flow and it is not known whether it measures total skin blood flow or nutrient blood flow (Nilsson et al, 1980).

Transcutaneous oxygen estimation offers an alternative method of assessing skin blood flow. This relies upon the assumption that for gaseous exchange in tissues there must be a satisfactory circulation. This method was originally developed for neonatal intensive care units (Huch et al,

1974) but has recently been used to assess patients with peripheral vascular disease (Eickhoff and Engell, 1981; Hansen et al, 1984; Wyss et al, 1984). It has also been used to predict amputation wound healing (Ratcliff et al, 1984; Katsamouris et al, 1984). Despite encouraging results with this technique the transcutaneous oxygen level can be influenced by other diseases apart from peripheral vascular disease. In particular cardiopulmonary pathology interferes with the results although this is partially overcome by comparing the values from different regions of the body (Eickhoff and Engell, 1981).

1.3.4 Radioisotopic Methods

These methods involve either the intravascular injection of radio-labelled materials or local injection of freely diffusible isotopes into the skin. The former method has been studied by Rhodes et al (1976) who injected radioactive isotopes intravenously and then scanned the area under study to assess blood flow. Lawrence et al (1983) used this technique to assess the healing potential of ischaemic leg ulcers. The amount of activity around the

ulcer was found to correlate well with ulcer healing. This technique has not received wide acceptance because it involves intravascular administration of high levels of radioactive material, expensive equipment only available in nuclear medicine departments and requires highly trained staff.

On the other hand the use of washout of local injections of radioactive isotopes is used widely experimentally, and to a lesser extent clinically, to measure skin blood flow. Lassen et al (1984) stated that this method is the only satisfactory method for measuring blood flow in human skeletal muscle, cutaneous and subcutaneous tissue. This method, which has been used in the present work, will be discussed in detail.

Isotope Washout Technique

The isotope washout technique was first described by Kety (1948) who related the rate of washout of the radioactive isotope Na-24 from muscle tissue to the blood flowing through the tissue. Kety (1951) discussed the

theoretical basis and applications of this technique in the measurement of tissue blood flow. The technique involved injection of a solution of the isotope in normal saline directly into the tissue under study. The washout of the isotope from the tissue is recorded by a detector placed above the injection site. The blood flow through the tissue can then be determined by a rearrangement of Kety's original equation (Kety, 1949; 1951) which becomes

$$f = k \cdot \lambda \cdot 100\text{mls} \cdot 100\text{g}^{-1} \cdot \text{min}^{-1}$$

where f = blood flow

k = washout rate constant

λ = tissue to blood partition coefficient for the isotope.

This theory relies on a number of assumptions (Hyman, 1960) which are

- (i) the material injected should be freely diffusible between the tissue and the blood,
- (ii) the material injected should cause no reaction or modification of the tissue under study,
- (iii) the injection should cause minimal distortion of the tissues,

- (iv) detection methods should be insensitive to minor re-distribution effects in the tissues and yet be sufficiently collimated to be insensitive to radio-activity in any area other than the injected depot, and
- (v) the sensitivity of the detector, and the activity of the sample must provide high counting rates to make possible valid determination of the quantity of label at the depot at relatively short intervals so that rapid estimations of the circulation can be determined.

The first three of these assumptions have been extensively investigated, and largely overcome, in the last two decades. Kety (1948) used Na-24 to estimate tissue blood flow but the clearance of this isotope was found to be diffusion limited. That is, the amount of Na-24 diffusing across the capillary would reach an upper limit despite the potential for greater capillary blood flow, thus making this isotope unsuitable for blood flow measurements. This problem has been overcome with the use of freely diffusible isotopes of the inert gases krypton-85 and xenon-133 (Lassen

et al, 1964; Holzman et al, 1964; Larsen et al, 1966). Xenon-133 has been widely used in the measurement of blood flow in skeletal muscle (Lindbjerg, 1967; Sejrsen and Tonnesen, 1968; Neubauer, 1977) and adipose tissue (Nielsen, 1972; Henriksen et al, 1973; Henriksen and Kristensen, 1979; Bjerre-Jepsen, 1982). This isotope is also suitable for cutaneous tissue blood flow measurement but is complicated by its diffusion into the underlying subcutaneous tissue (Sejrsen, 1969).

Recently another isotope, technetium-99m, has been used to measure skin blood flow (Linde and Hjemdahl, 1982; Young and Hopewell, 1983; Faris and Lassen, 1982). Tc-99m is a product of molybdenum-99 and is generated as the pertechnetate ion in isotonic saline solution. The isotope has a half life of 6 hours (Miller, 1975). The advantage of Tc-99m over Xe-133 is that the latter isotope is highly lipophilic resulting in its accumulation in fatty tissues. This has complicated its use when measuring skin blood flow as the isotope diffuses into the fatty subcutaneous tissue thus interfering with blood flow estimates (Sejrsen, 1969).

A criticism of the use of Tc-99m was that it may be diffusion limited thus giving falsely low flow results at high flow rates (Lassen and Holstein, 1974; Snelling et al, 1980). However, some workers suggest that the flow rates in the skin are not sufficiently high for diffusion to be a limiting factor (Challoner, 1972; Dunjic, 1974). Linde and Hjemdahl (1982) and Holstein et al (1983) have recently confirmed that Tc-99m is a suitable isotope for blood flow estimations in cutaneous tissue, even under conditions of maximum flow.

The other major problem associated with the isotope washout technique is the tissue trauma associated with the injection of the isotope. The injection trauma results in a hyperaemia giving a high blood flow (Larsen et al, 1966). This can be overcome to a large degree by an atraumatic labelling of the tissue with the Xenon-133 (Sejrsen, 1971). This problem of injection-induced hyperaemia is not important if the vessels under study are dilated as occurs when studying the maximum blood flow under conditions of reactive hyperaemia or injections of vasodilating agents.

The isotope washout technique, providing one is aware of its limitations, enables an objective, accurate measure of capillary blood flow through the skin. At present there is no other method to obtain this information. In this study we have used this technique to measure skin blood flow, vascular resistance and skin perfusion pressure.

OBJECTIVES

The objectives of this thesis are

- (i) to develop a clinical test specific for skin microvascular disease of the lower limb,
- (ii) to determine, in an animal model, the vasodilator most proficient at dilating the skin microvessels,
- (iii) to investigate the significance of microvascular disease, in conjunction with large vessel disease, in the pathogenesis of lower limb ischaemia.
- (iv) to examine the usefulness of measuring parameters of large vessel and microvessel disease in the prediction of healing of ischaemic ulceration and gangrene of the lower limb and to use this information to construct a rational management plan for these patients.
- (v) to construct a mathematic model to determine the importance of age, sex, the presence of diabetes and hypertension and microvessel disease in the healing of lower limb ischaemia.

CHAPTER 2

ISOTOPE WASHOUT TECHNIQUE TO MEASURE SKIN PERFUSION PRESSURE AND VASCULAR RESISTANCE

INTRODUCTION

Nilsen et al (1967) introduced the concept of measuring the blood pressure in skeletal muscle by determining the amount of external pressure over the muscle required to stop the washout of Xe-133. Holstein and Lassen (1973) used this method to measure the local perfusion blood pressure in the skin. They used the isotope I-131 bound to antipyrine (I-131-ap). This isotope, unlike Xe-133, is not highly lipophilic and therefore does not diffuse into the underlying fatty subcutaneous tissue. The I-131-ap was injected with histamine which dilated the vessels under study thus increasing blood flow and simplifying the interpretation of the effect of external pressure on flow. They found that the local perfusion pressure measured in this way corresponded to the diastolic pressure in the arm.

The method of determining the arm pressure in this study was not stated. Holstein et al (1977; 1980) compared the skin perfusion pressure (SPP) with directly recorded femoral artery pressure and demonstrated that the SPP was slightly lower than the mean arterial pressure.

Holstein (1973) and Holstein et al (1979) used this method of measuring SPP in patients with peripheral vascular disease who were having amputations. They found that the SPP was a reliable indicator of healing of the amputation site. This method has not received wide acceptance in clinical practice possibly because it involves expensive equipment, and the injection of radioactive isotopes. We have used an adaption of this method (Faris and Lassen, 1982) which not only simplifies the measurement of SPP but also provides information about the skin vascular resistance. This method will now be discussed in some detail.

METHOD

The method used in this study involved an intradermal injection, via 26g needle, of 0.05mls Tc-99m containing 250microCi. The injection was made as shallow as possible so that a pale bleb was raised. Injected with the Tc-99m was 0.1-0.15mls of either histamine (50microg) or nitroprusside (250-300mg) in normal saline. These agents dilated the vessels under study, thus eliminating any local or neurological factors which may influence local blood flow. The injection was placed into either the dorsum of the foot or the antero-medial aspect of the calf, midway between the medial malleolus and the patella. The gamma-emission of the isotope was counted by a NaI scintillation detector placed 10-20cm from the depot. The number of counts each 10 seconds were recorded by a scaler-ratemeter (SR 7, Nuclear Enterprises Ltd, UK) set at the 141kV peak for Tc-99m. An on-line Apple II microcomputer was programmed to calculate the line of best fit by the least squares method. The slope of this line represents the rate of washout of the isotope and can be represented as:

$$k = \frac{\ln C_1 - \ln C_2}{t_1 - t_2}$$

where C_1 represents the number of counts from the isotope depot at time 1 (t_1) and C_2 the counts at time 2 (t_2). $k \text{ min}^{-1}$ is the washout rate of the isotope from the depot. Referring to the general equation formulated by Kety (1951)

$$f = k \cdot \lambda \cdot 100\text{mls.} \cdot (100\text{g min})^{-1}$$

where f = flow

k = washout rate of isotope

λ = partition coefficient for Tc-99m between blood and the tissues.

Therefore, it can be seen that the washout rate k is directly proportional to the blood flow.

To determine the skin perfusion pressure (SPP) and skin vascular resistance (SVR) external pressure was applied over the depot by means of an air filled plastic bag (8 x 10cm) held in position by a standard sphygmomanometer cuff (fig. 2.1). The plastic bag was filled with sufficient air to

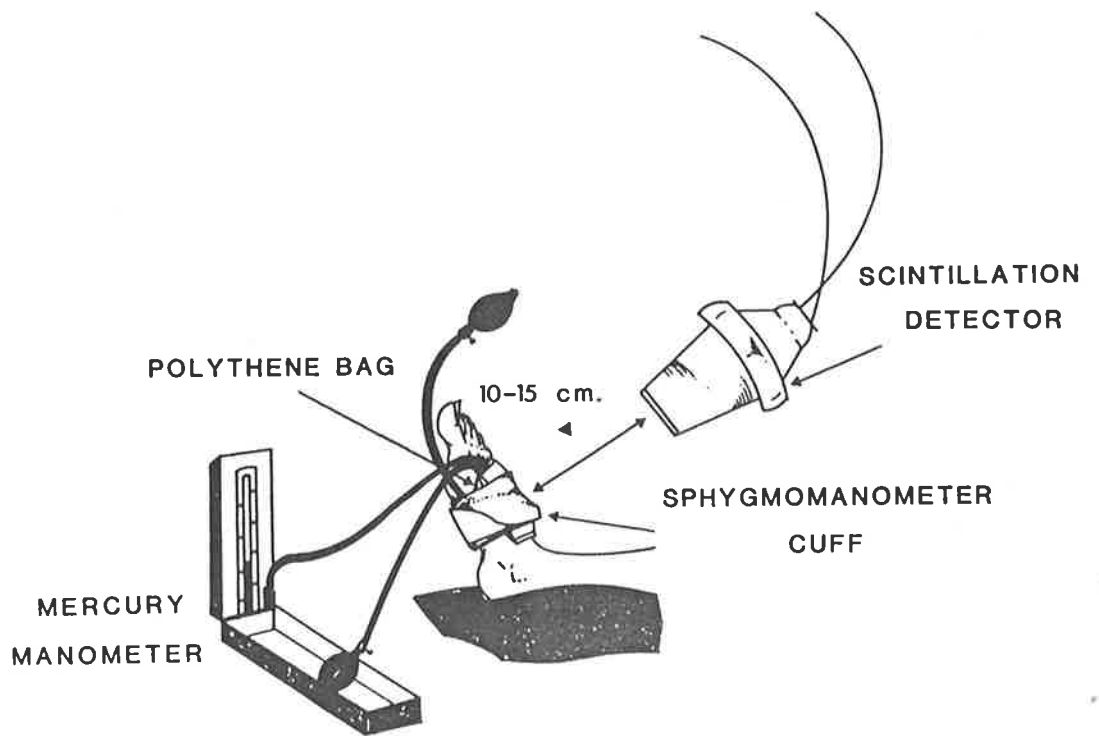


FIGURE 2.1

Illustration of equipment used to measure
SPP and SVR.

enable it to mould smoothly over the labelled area so that the pressure was applied evenly over the depot and adjacent areas. This is important as a blood pressure cuff by itself will wrinkle on the inner surface resulting in uneven pressure on the skin (Holstein et al, 1977). The pressure in the bag was recorded by a mercury manometer connected directly to the bag and was varied by changing the pressure in the sphygmomanometer cuff by a hand held pump.

The external pressure (PmmHg) was increased in a stepwise fashion by 10-20mmHg until the washout rate approached zero (fig 2.2) A new washout rate was determined at each pressure. The external pressure was returned to zero and the procedure repeated. The relationship between the washout rate and applied pressure was found to be linear (Faris and Lassen, 1982). The line $k = bP + a$ was determined by the least squares method (fig 2.3). The intercept with the vertical axis, a , was the washout rate at zero external pressure. This is, therefore, the maximum washout rate, k_{max} , which is directly proportional to the maximum skin blood flow. The slope of the line, b , is the change in washout rate for a given change in external pressure.

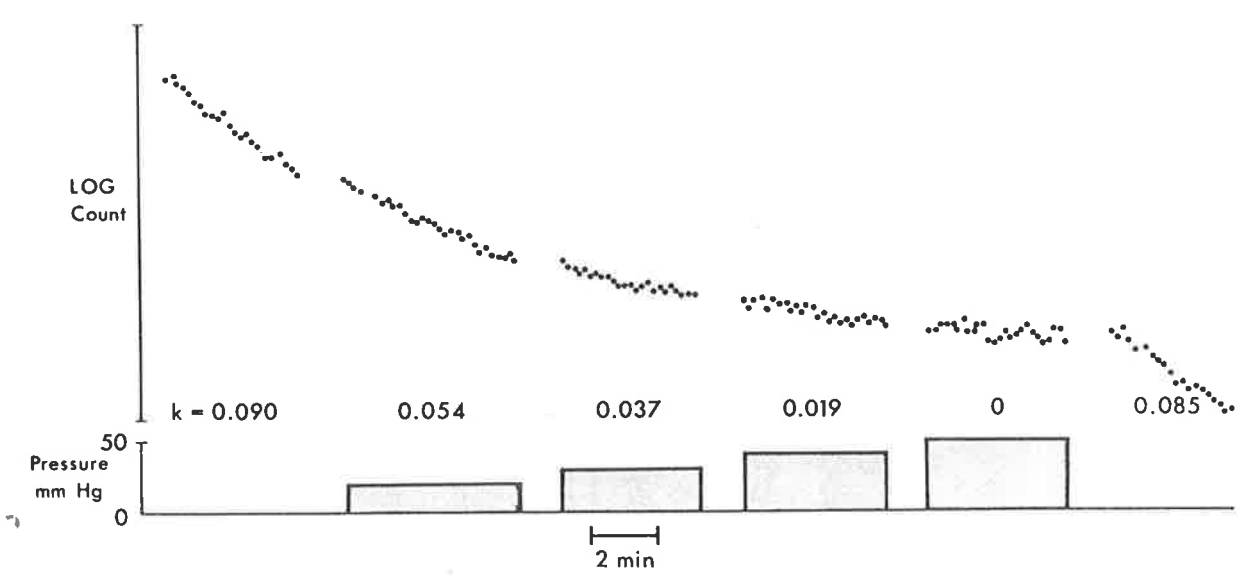


FIGURE 2.2

Radioactivity in the injected depot at each level of external counter pressure (shown at bottom of figure). The washout rate (k) at each pressure is shown.

(From Faris and Lassen, 1982).

CALCULATION OF VASCULAR RESISTANCE AND PERFUSION PRESSURE

In a maximally dilated vascular bed the blood flow will be determined by the perfusion pressure and the resistance of the arterioles, capillaries and venules. In the technique described above the perfusion pressure was altered in a controlled fashion by means of external pressure. By determining the amount of change in the flow, or washout rate, for a given change in pressure the vascular resistance can be determined. This can be expressed mathematically as:

$$\text{resistance} = \text{pressure}/\text{flow}$$

Referring to figure 2.3 the slope of the line, b , is a ratio of flow to pressure. That is, the inverse of the vascular resistance. Therefore, the inverse of the slope, $1/b$, is a measure of the resistance. As the vessels are maximally dilated, this represents the minimum skin vascular resistance.

The intercept of the line (fig 2.3) with the pressure axis, a/b , is the pressure at which the washout rate becomes zero. This is the skin perfusion pressure (SPP). This method differs from previous methods in that this is a

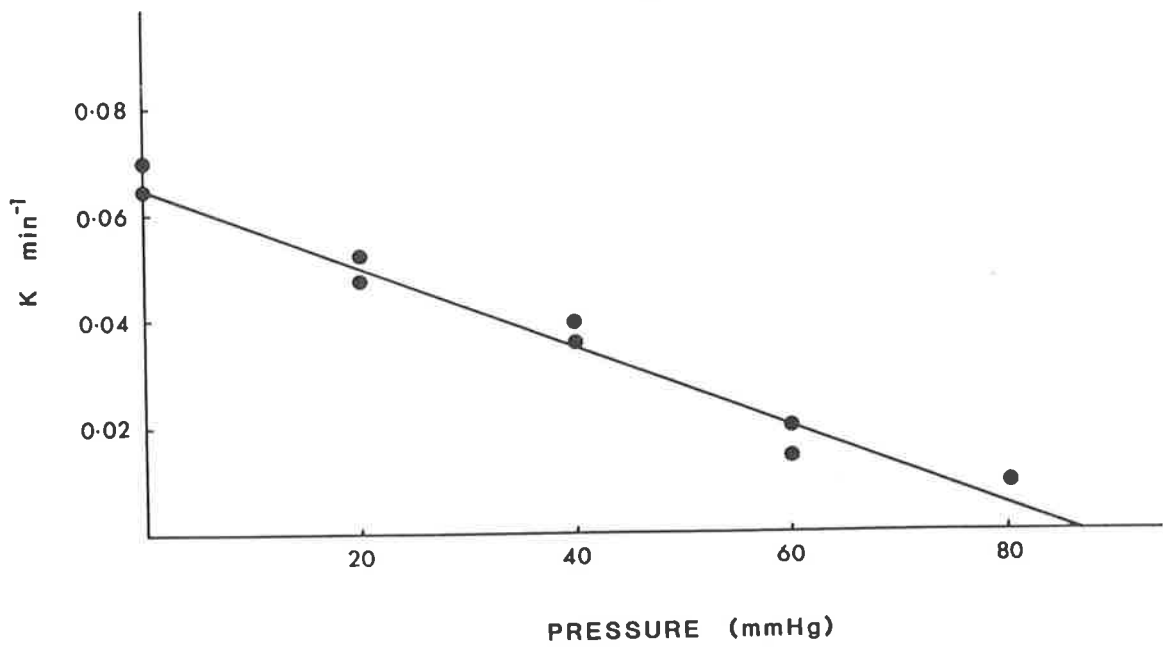


FIGURE 2.3

Relationship between external pressure and washout rate (k). The line is calculated line of best fit (see text).

calculated pressure not the actual pressure observed to stop washout. This is an advantage as it is often difficult to determine exactly at what pressure the washout of the isotope ceases. This applies especially to the situation when Tc-99m is being used as the natural decay of this isotope is quite rapid (half life 6 hours) thus making the interpretation of low washout rates difficult (Holstein et al, 1983).

This method is simple, requires inexpensive equipment, takes 20-30 minutes for completion and is well tolerated by almost all patients. In fewer than 5% of patients the study cannot be completed. This is almost always due to movements in patients with advanced cerebrovascular disease.

CHAPTER 3

ANIMAL STUDY TO DETERMINE SUITABLE VASODILATOR

INTRODUCTION

The production of a state of maximum dilation in the vascular bed being studied is very important. When the vessels are maximally dilated they behave passively. That is, the blood flow varies directly with the perfusion pressure. In these circumstances the vascular bed is considered to be free of local, humoral and neurological controls.

Paralysing the vascular bed by ischaemia or ischaemia plus exercise is the most common method of creating a maximally dilated vascular bed (Hartling et al, 1978; Faris et al, 1982). Ischaemia or ischaemia plus exercise are, however, uncomfortable for the patient, may alter central haemodynamics and may not be effective in dilating the cutaneous vascular bed.

Some authors have, therefore, used local injections of vasodilating agents to produce vasodilation. Henriksen and Kristensen (1979) used papaverine to study the distensibility of the vascular bed in human subcutaneous tissue. Faris et al (1982), also using papaverine, demonstrated a decreased distensibility of the vessels in skeletal muscle of diabetic patients. Faris and Lassen (1982) demonstrated an increase in the vascular resistance in vasodilated cutaneous tissue of diabetic patients. These authors used histamine to cause this vasodilation.

The critical assumption made by these workers was that the vascular bed under study was maximally dilated but there is little evidence to suggest that either papaverine or histamine, at the doses used in these studies, produced this critical maximum vasodilation.

It was the aim of this study to use an animal model to:

- (i) determine dose response curves for histamine, papaverine, nitroprusside, verapamil and prostaglandin -E₁

- (ii) compare the effectiveness of the five agents
at their maximum doses to produce vasodilation,
- (iii) assess the extent of oedema caused by the agents,
and
- (iv) examine the duration of response to each agent.

METHODS

1. General Methods

Lop-eared rabbits weighing 1500-4000 g were anaesthetised by intravenous sodium phenobarbitone (12-30 mg/kg), injected via a 21 g butterfly needle inserted into an ear vein. In approximately 50% of the rabbits studied an intra-arterial catheter was inserted into an ear artery and connected to a pressure transducer (P23 ID, Statham Instruments Div, Gould Inc. Ca, USA). The blood pressure and heart rate were recorded by a physiological recorder (Physiograph Mk-IV, Narco, Bio-Systems, Tx, USA). The rabbit was placed in the prone position and shaved to expose the dorsal skin. Room temperature was constant during all studies in the range 20-24°C.

An injection containing 0.1 ml of a freely diffusible isotope, either Xe-133 or I-131-antipyrine (I-131-ap), and 0.2 ml of a test substance was made into a subcutaneous depot on the dorsum of the rabbit (fig. 3.1). The gamma emission was detected by a NaI scintillation detector placed

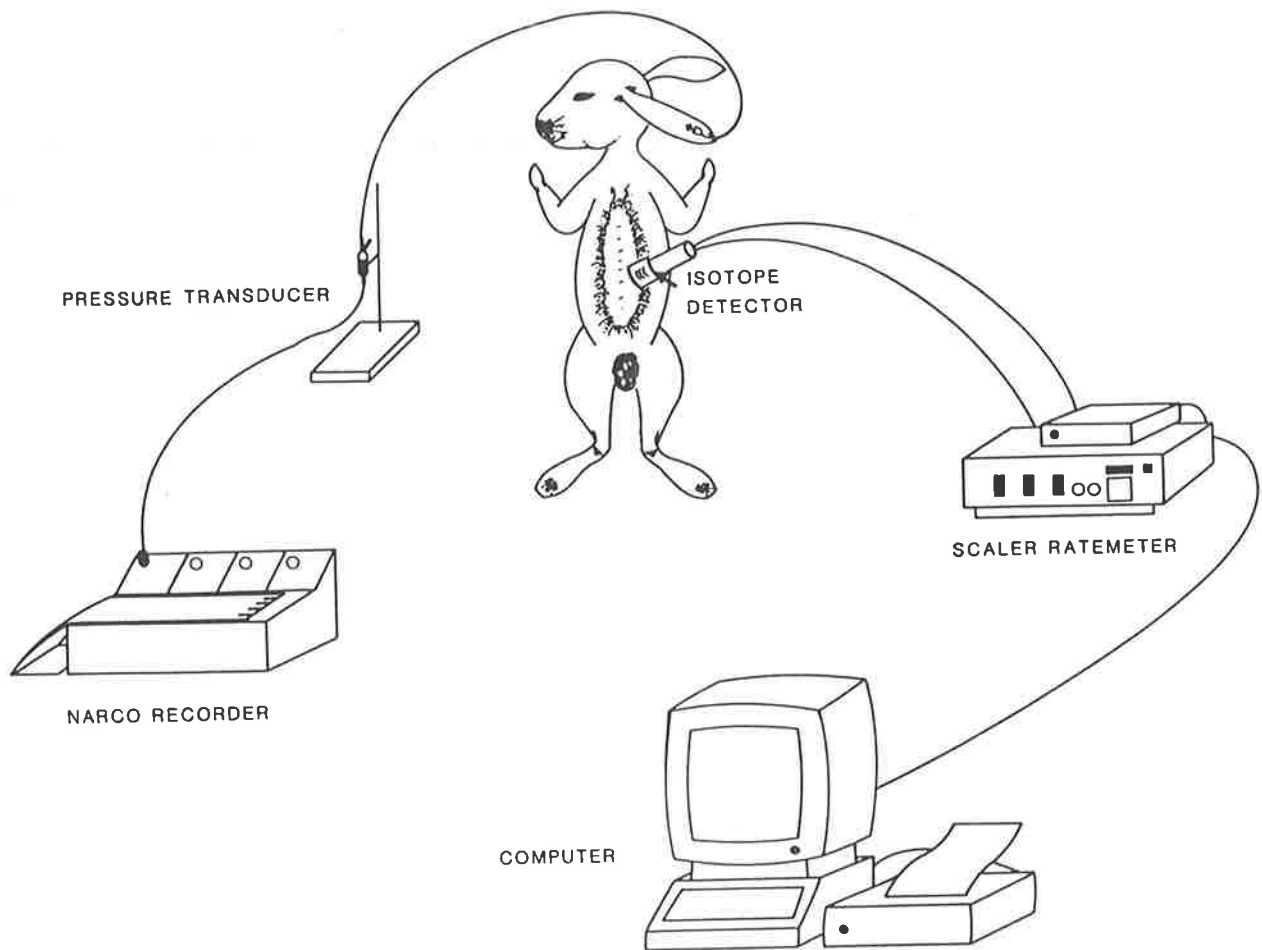


FIGURE 3.1

Experimental design to determine subcutaneous tissue washout rates and direct blood pressure in rabbits.

5-10 cm from the subcutaneous depot. Lead shielding was used to eliminate interference from other depots. Background counts were determined from the hindquarter of the rabbit and appropriate adjustments made in the calculations. Counts were determined every 10 seconds for 3 minutes by a scaler rate meter (SR7, Nuclear Enterprises Ltd, Reading, UK) set around the 81 KeV peak for Xe-133 or 360 KeV for I-131. An on-line Apple II microcomputer displayed a plot of the natural logarithm of the number of counts each 10 sec against time. The computer was programmed to determine the slope of this line by the least squares method. This represents the washout rate constant ($k \text{ min}^{-1}$).

2. Determination of Dose-Response curves

Dose-response curves were obtained for histamine, papaverine, nitroprusside, verapamil and prostagandin E1. Xe-133 (Amersham XAS, 120P, Amersham Aust, Aust) containing approximately 100 micro Ci, was the radioactive isotope used in this experiment. Five subcutaneous depots were used in each rabbit enabling a comparison of control (isotonic

saline) and four doses of one test agent. Ten rabbits were used for each agent. Preliminary studies were performed to determine the approximate doses required to produce maximum vasodilation.

The washout rate ($k \text{ min}^{-1}$) from each depot was determined at 20, 24 and 28 minutes after the injection. This was to avoid the traumatic hyperaemia secondary to the injection (Nielsen, 1972). The mean of the three k values from the ten rabbits were then averaged to give an average washout rate (k_{av}) which was used to construct the dose-response curves.

3. Comparison of maximal doses

The aim was to compare the five agents with each other at their most effective doses as determined in the first experiment. The experimental design was identical to the previous experiment, except that instead of injecting different doses of one agent into the rabbit maximum doses of each agent were injected. Ten rabbits were studied.

4. Oedema formation

Oedema formation was assessed by comparing the affect of the vasodilating agent on the washout of two isotopes with different characteristics. The isotopes used were Xe-133 and I-131-antipyrine (I-131-ap).

The I-131-ap was not commercially available and was prepared in our laboratory by Mr. Neville De Young by the method described by Robinson and Lee (1979).

Unlike Xe-133, I-131-ap is not eliminate with passage through the lungs. This resulted in distribution of this isotope through the tissues resulting in high background activity, making a comparison of more than two agents in one rabbit difficult. It was, therefore, decided to test nitroprusside, which was the most potent vasodilator in the previous experiment, with the widely used agents histamine and paraverine in separate sets of five animals.

The washout rate ($k \text{ min}^{-1}$) was determined at 20, 24 and 28 minutes and were then averaged (k_{av}). There results were

compared with the kav obtained with Xe-133 in the previous experiment.

5. Duration of Response

In the experiments using I-131-ap the washout rates were also determined each four minutes from the time of the injection for 28 minutes.

6. Histological studies

Histological studies were made from three depots in which nitroprusside injected 7-10 days previously to assess any detrimental effect this agent may have on the local tissue. Cutaneous and underlying subcutaneous tissue down to the deep fascia was removed under general anaesthesia and immediately placed in formalin. The specimens were then impregnated with paraffin and sectioned in 2 micro m thicknesses, stained with haematoxylin and eosin and were assessed for the presence of inflammatory cells and fibrosis.

7. Statistics

All results were expressed as mean \pm 1 SEM and analysed by the paired t-test.

RESULTS

1. Dose response Curves

The dose response curves are shown in figures 3.2 - 3.6. The doses which produced greatest washout were 6 micro g for prostaglandin-E₁, 0.5 mg for verapamil, 60 micro g for nitroprusside, 5 mg for papaverine and 100 micro g for histamine. The levels of significance are shown in the figures. With each agent there was a large variation in the k values between rabbits of up to 150%, but the within-rabbit response was constant. This explains the large standard error seen. At no stage did the local injection alter the blood pressure or heart rate.

2. Comparison of maximal doses

The second experiment allowed a direct comparison of the maximal vasodilator action of the different agents. The results are shown in figure 3.7. Nitroprusside was used as the reference agent with which the other agents were compared as it appeared to be the most effective. The k_{av}

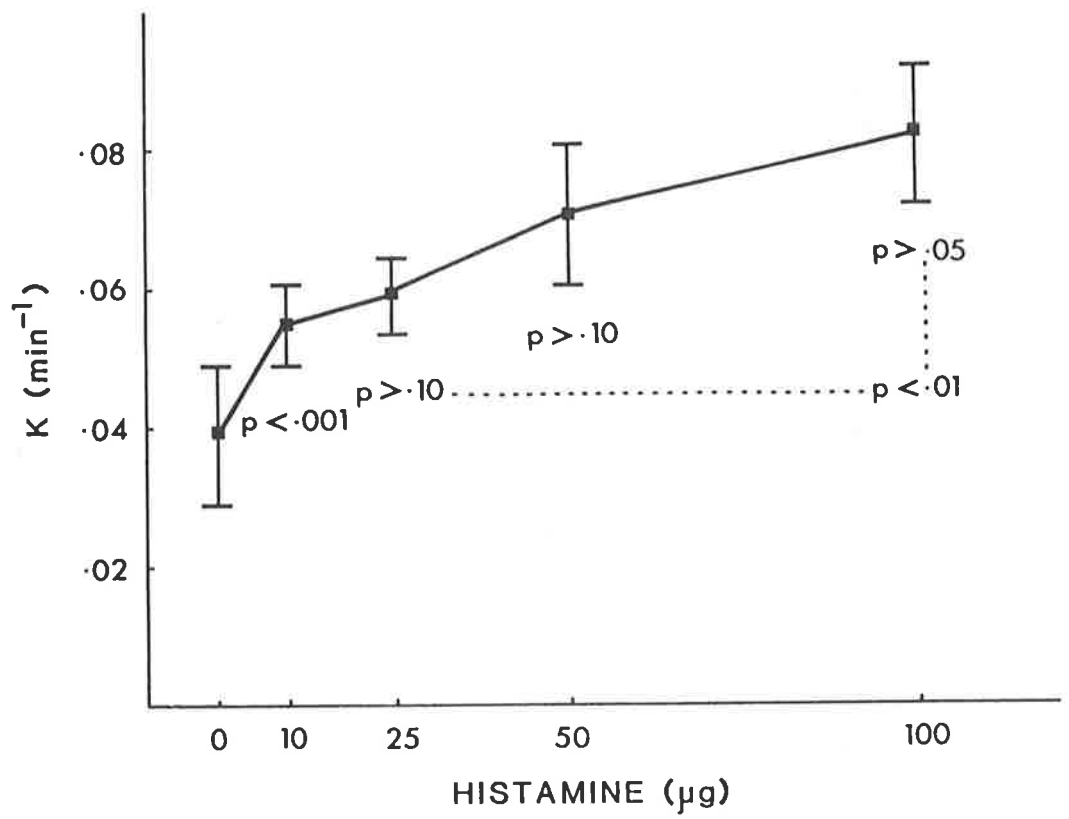


FIGURE 3.2

Dose-response curve for histamine.

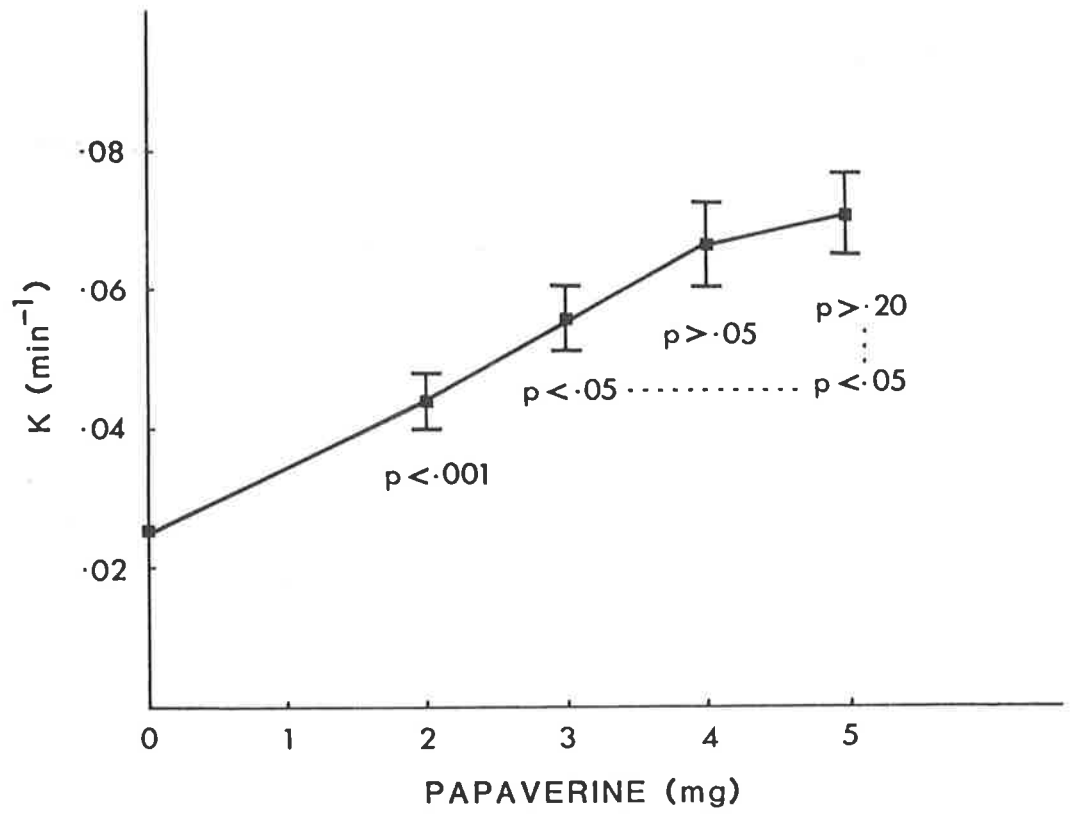


FIGURE 3.3

Dose-response curve for papaverine.

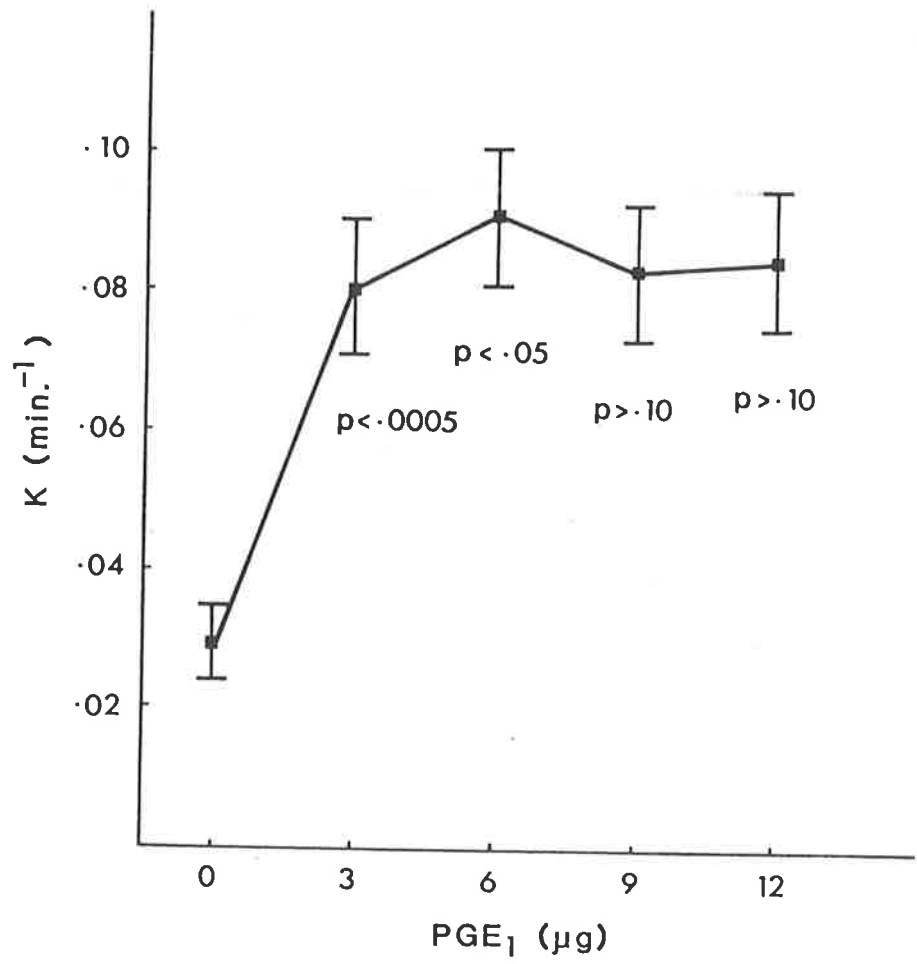


FIGURE 3.4

Dose-response curve for prostaglandin-E₁.

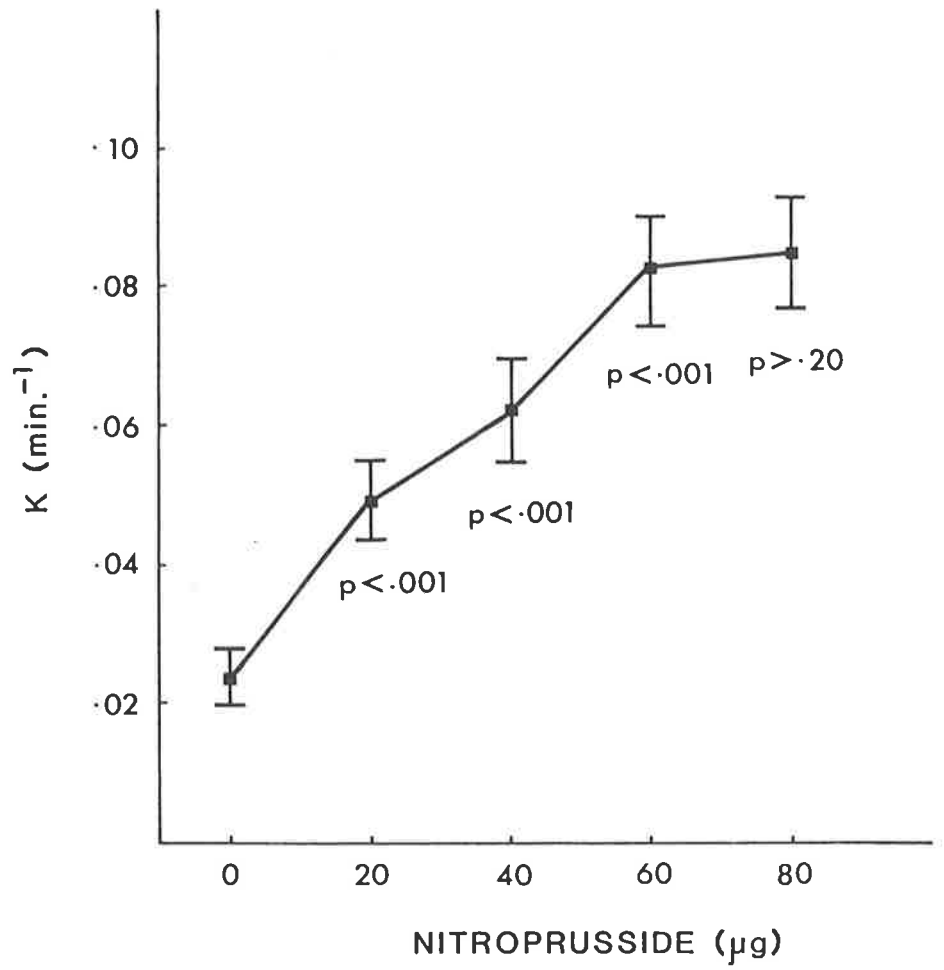


FIGURE 3.5

Dose-response curve for nitroprusside.

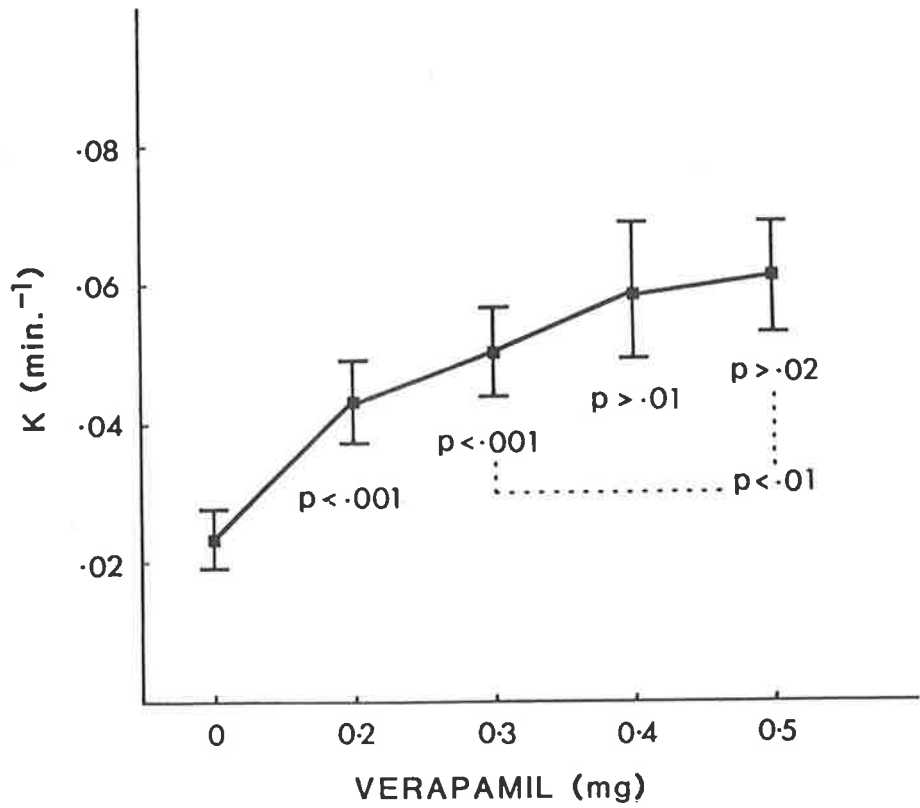


FIGURE 3.6

Dose-response curve for verapamil.

(± SE) for the nitroprusside was 0.1140 (± 0.013 min⁻¹). For papaverine the kav was 0.0750 (± 0.0160 min⁻¹). This was significantly lower than nitropursside (p < .01). kav for histamine (0.0540 ± 0.013) and varapamil (0.0785 ± 0.0143 min⁻¹) were also lower than nitroprusside (p < .001 and p < .01 respectively). On the other hand PG E1 (0.1040 ± 0.0180 min⁻¹) was not different to nitroprusside (p > .20), verapamil (p > .10) or papaverine (p > .20). PG E1, however, produced a higher kav than histamine (p < .01). Nitroprusside, therefore, produced a greater increase in kav for Xe-133 than paraverine, varapamil and histamine, whereas PG E1 produced a greater kav than histamine but not the other agents tested.

3. Comparison of washout rate of Xe-133 and I-131-ap

With I-131-ap as the diffusible isotope the kav for nitroprusside (0.1043 ± 0.0106 min⁻¹) was similar to histamine (0.0816 ± 0.0172 min⁻¹; p > .10) but was higher than papaverine (0.0791 ± 0.0096 min⁻¹; p < .05). This is a contrast to the results obtained when ¹³³Xe was used as the kav for nitroprusside was higher than both histamine and

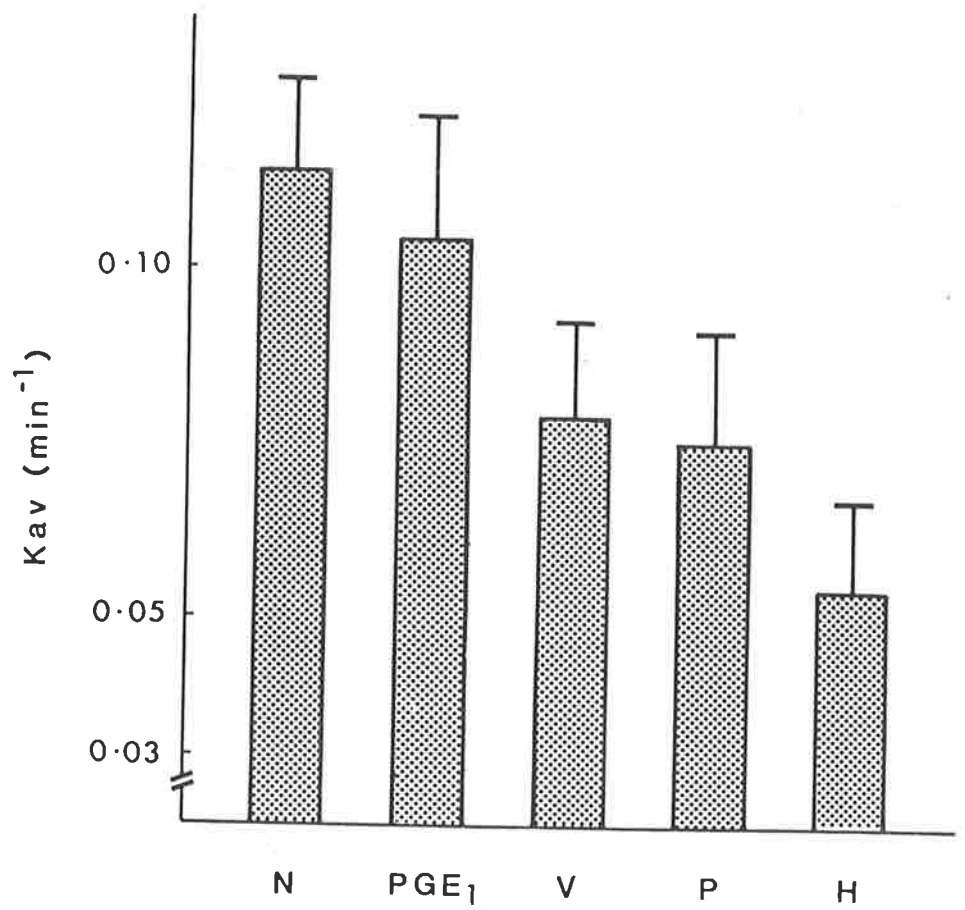


FIGURE 3.7

Comparison of the maximum doses of nitroprusside (N), prostaglandin E₁ (PGE₁), verapamil (V), papaverine (P) and histamine (H).

papaverine (fig. 3.8).

4. Duration of Response

The duration course of the response is demonstrated in figure 3.9. Nitroprusside is more effective than papaverine and histamine for the 30 minutes tested, although this loses significance for histamine after 16 minutes. The other interesting finding is that histamine appears to take 16 minutes to reach maximum effectiveness, whereas nitroprusside and papaverine have their maximum effect within the first four minutes.

5. Histological Studies

The histological studies were normal indicating that nitroprusside had no direct detrimental effect on the tissue.

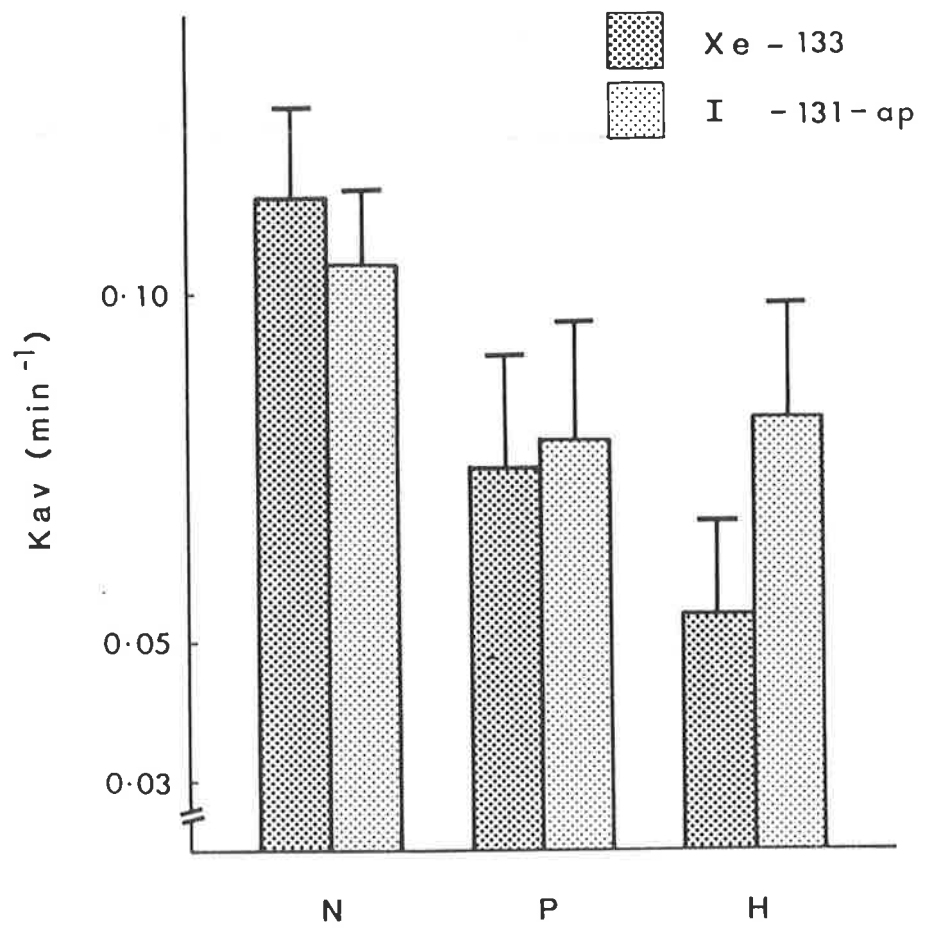


FIGURE 3.8

Comparison of Xe-133 and I-131-ap washouts for nitroprusside (N), papaverene (P) and histamine (H).

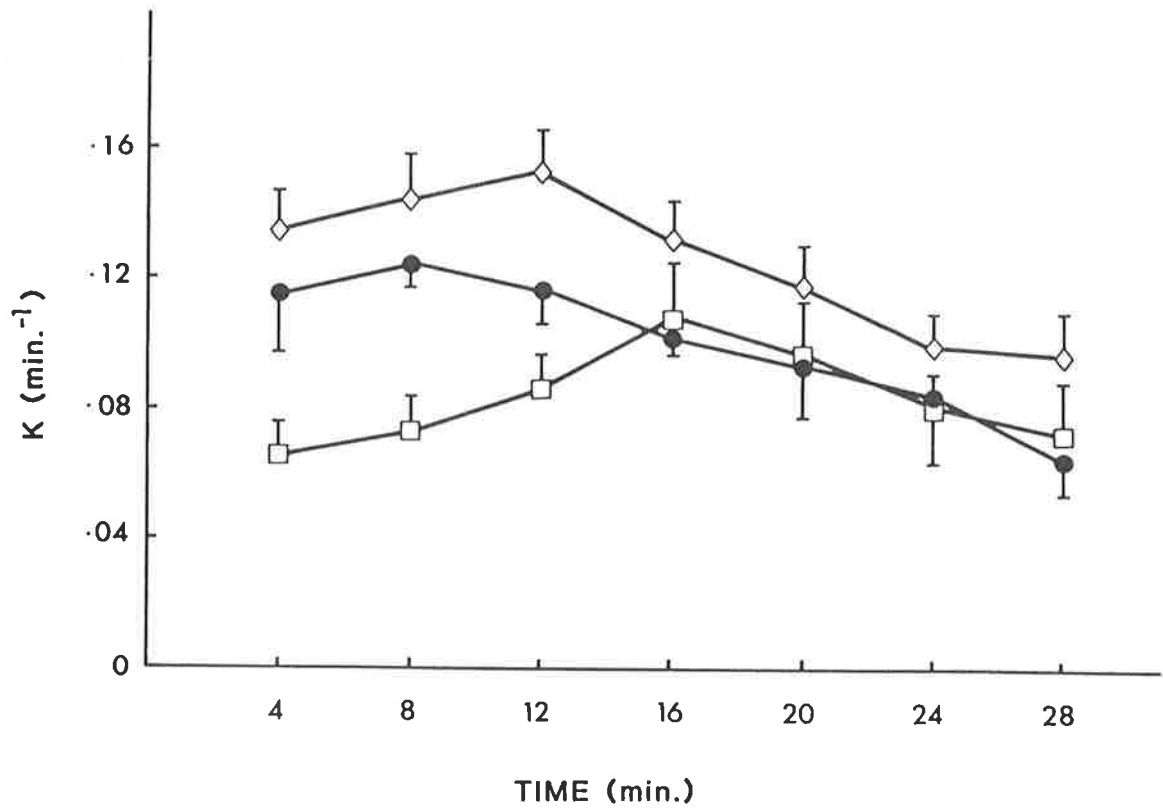


FIGURE 3.9

Time course of action of nitroprusside (◇),
histamine (□) and papaverene (●).

DISCUSSION

The results from the first two experiments clearly demonstrate that nitroprusside produced greater changes in the washout rate than either papaverine, histamine or verapamil. A number of variables must be considered, however, before a definite conclusion can be made concerning the actual degree of vasodilation produced by each agent.

Xe-133 was used as the diffusible isotope for a number of reasons. It is the most widely used isotope for estimation of both subcutaneous and cutaneous blood flow (Larsen, Lassen and Quaade, 1966; Sejrsen, 1969; Henriksen, 1973; Lindbjerg, 1967). Xe-133 is a freely diffusible inert gas which means that most of the Xe-133 is eliminated from the blood with passage through the lungs resulting in very low recirculation of Xe-133 and, therefore, low background activity. This is important as each rabbit was receiving five separate injections of Xe-133 over a 150 minute period. If the isotope was not eliminated by the lungs it would accumulate in the body tissues resulting in high background activity, thus making interpretation of the result

difficult. When Xe-133 was used background activity was always less than 10% of the total counts for each depot. On the other hand I-131-ap, which is not eliminated by the lungs, accumulated in the tissues, resulting in high background activity of the order 30-50% of the count rate if the rabbit was subjected to 3 or 4 injections. The other advantage is that Xe-133 gas in solution is readily available commercially.

Despite these advantages Xe-133 has its own inherent problems. To estimate the actual blood flow through the tissue in question, it is possible to use a rearrangement of Kety's original equation (Kety, 1949 and 1951) which is $f = k \cdot \lambda \cdot 100$ (see chapter 2). Xe-133 is a highly lipid soluble compound resulting in a partition coefficient that is very dependent on the amount of fat and tissue fluid present (Bjerre-Jepsen et al, 1982). A wide variation in fat content between rabbits makes it difficult to compare responses between rabbits to the same agent without estimating the partition co-efficient in every case. This problem was overcome by using each rabbit as its own control. That is, different doses of one agent and the

control substance were tested in the same rabbit. It was assumed that there was relatively small variation in the partition co-efficient between different anatomical regions on the dorsum of the rabbit compared to the same region between different animals. The inter-regional variation was further reduced by randomly allocating the particular injection to the different anatomical regions.

The effect of changes in tissue fluid on the partition coefficient for Xe-133 is more difficult to overcome. It is possible for the vasodilator to increase tissue fluid by either increasing capillary hydrostatic pressure, resulting in a change in the forces which regulate fluid movement across a capillary membrane with an increase in interstitial fluid, or it may alter capillary permeability directly.

To assess the effect of tissue fluid on the partition coefficient for Xe-133 we compared the washout rates for Xe-133 and I-131 bound to antipyrine (I-131-ap). I-131-ap was used because its partition coefficient is close to unity and thus independent of the fat content of tissue (Lindbjerg, 1967).

The washout rate for both isotopes at a constant capillary blood flow depends on the capillary density as well as the partition coefficient. If the tissue volume increases due to oedema then there is a corresponding decrease in the capillary density and, therefore, an equivalent decrease in the washout rate provided the partition coefficient remains constant as occurs with I-131-ap. However, the partition coefficient for Xe-133 will be reduced with oedema formation thus increasing the washout rate. Therefore, when Xe-133 is used to measure blood flow, oedema will tend to influence the washout rate in two opposite directions. The decrease in capillary density reducing the washout rate and the decrease in partition coefficient increasing the washout rate. When comparing the results of Xe-133 and I-131-ap washout rates for each vasodilator in each depot, it can be assumed that the capillary density will change to the same extent and thus will have equivalent effect on the washout rates of both isotopes. That is, any difference in washout rates between two isotopes for any one vasodilator is mainly due to alteration in tissue fluid content.

From figure 3.8 it can be seen that the ratio of the washout rates for Xe-133 and I-131-ap (i.e. k_{Xe}/k_{I-ap}) was much lower for histamine than either nitroprusside or papaverine. This implies that histamine caused less oedema than either nitroprusside or papaverine. Despite the oedema produced by the nitroprusside the maximum washout rate was greater than either other agent thus supporting the conclusion that it was the most potent vasodilator tested. If a hydrophilic isotope such as I-131-ap or Tc-99m is used this tissue fluid might be expected to have only a small effect on the results, but if a lipid soluble isotope such as Xe-133 is used then there would be a large effect.

The other significant finding is that although nitroprusside has a very short biological half life intra-arterially, this does not appear to be the case when injected subcutaneously as it was still causing greater vasodilation than either papaverine or histamine after 30 minutes. This is not surprising as nitroprusside is broken down by the red blood cells (Gilman, 1980) and will not be inactivated until absorbed from the tissue into the blood.

This is important as some studies on the microcirculation may take up to 30 to 45 minutes for completion. It is interesting to note that both nitroprusside and papaverine reach their maximum effect within four minutes whereas histamine takes up to 16 minutes. The reason for this is not clear.

Nitroprusside is normally used as an intravenous infusion to lower blood pressure in a variety of clinical situations. The doses used in these situations are 100-500 times the doses we used to local injections. Histological studies of the injection sites in the rabbits showed no evidence of inflammation or fibrosis, suggesting that local injections do not cause tissue damage.

Nitroprusside has other advantages over papaverine apart from being a more potent vasodilator. The appropriate dose of nitroprusside can be injected in very small volumes whereas this is not possible for papaverine, as 5 mg of papaverine requires at least 0.2 ml of solvent. This is important with intradermal injections where it is physically difficult to give injections of larger volumes. Larger

volumes may also increase local tissue tension thus interfering with capillary blood flow. Nitroprusside is more convenient than histamine as histamine injections are painful, thus being not only unpleasant for the patient, but also causing a generalised increase in sympathetic output and therefore alterations of the central haemodynamics.

This study demonstrated that nitroprusside is a potent vasodiator when injected directly into localised vascular beds, and that the doses used are small and should not cause any systemic effects or local tissue damage.

CHAPTER 4

EVALUATION OF ISOTOPE WASHOUT TECHNIQUE TO MEASURE SKIN VASCULAR RESISTANCE AND SKIN PERFUSION PRESSURE

INTRODUCTION

The assessment of blood supply of the foot and lower limb is important in the management of patients with peripheral vascular disease, in particular, patients with distal gangrene and ulceration. The various methods used to assess lower limb perfusion (segmental blood pressures, transcutaneous oxygen tension, laser Doppler, Doppler ultrasound and photoplethysmography) have been discussed in chapter 1. In chapter 2 an isotope washout method was described which enables the measurement of skin perfusion pressure (SPP) and skin vascular resistance (SVR). It was the aim of this study to evaluate this method in normal subjects and to determine its reproducibility, the effect of age, peripheral vascular disease, arterial surgery and regional variation. The ankle pressure and SPP were compared in a large group of diabetic and nondiabetic

patients to determine the prevalence of arterial rigidity.

METHODS

Subjects

The main group investigated consisted of 33 normal subjects and 15 patients with peripheral vascular disease. The normal subjects were divided into two groups depending on age. Group I were subjects less than 30 years of age. This group consisted of 20 individuals with a mean age of 20.4 (18-27) years. There were 11 men and 9 women. Group II consisted of 13 subjects with ages greater than 30 years. The mean age of this group was 58.3 (32-86) years and consisted of 6 men and 7 women. No subject in either group has a history of diabetes mellitus, hypertension or peripheral vascular disease, which was excluded by normal ankle pressures and palpable foot pulses. The median age of the 15 patients with peripheral vascular disease (Group III) was 67.9 (46-87) years. There were 8 men and 7 women, none of whom had a history of hypertension or diabetes. All patients had severe peripheral vascular disease requiring arterial reconstructive surgery.

The auscultatory arm blood pressure was measured in the left arm of all patients using a 12 x 26 cm cuff. The diastolic pressure was taken at the cessation of the Korotkoff sounds and the mean arterial pressure (MAP) was calculated as the diastolic pressure plus one third of the pulse pressure. Ankle pressure was determined in each patient by using a photoplethysmograph probe placed on the toe as a pulse detector. The SPP, SVR and kmax were determined as described in chapter 2 on the anteromedial aspect of the calf. Nitroprusside was the vasodilator used in this study.

Vascular resistance was also estimated (EVR) in groups I and II by dividing the arterio-venous pressure gradient by the washout rate calculated with no external pressure (Skagen and Henriksen, 1983). The venous pressure in the leg of a supine patient is very low and for simplification was taken as 0 mmHg. Therefore, the pressure gradient was assumed to be equivalent to the mean arterial pressure.

Reproducibility

The reproducibility of the test was determined in eight subjects with peripheral vascular disease. Their mean age was 65.5 (47-87) years and there were 5 men and 3 women. One patient had a history of hypertension, one of diabetes mellitus and another both diabetes and hypertension. The SPP, SVR and kmax were determined on the dorsum of the foot and was repeated on the following day.

Influence of location

The SPP, SVR and kmax were determined on the dorsum of the foot and on the arteromedial aspect of the calf in 12 patients to determine if location influenced any of these parameters. The mean age was 65.6 (18-86) years and there were 7 men and 5 women. Five patients were free of peripheral vascular disease. The remaining seven patients had peripheral vascular disease and were studied post-operatively. No patients had diabetes mellitus but two patients had a history of hypertension.

Influence of surgery

The effect of arterial surgery on the SPP, SVR and kmax on the dorsum of the foot was determined in 8 patients undergoing femoropopliteal bypass surgery. The median age of the group was 68 (46-82) years and there were 5 men and 3 women. Two patients were diabetics and two had hypertension. One patient had both diabetes and hypertension. The pre-operative test was taken 24-48 hours prior to surgery and repeated 7-10 days following surgery.

Comparison of ankle pressure and SPP

The SPP on the dorsum of the foot was estimated in 99 patients who were divided into two groups. There were 46 nondiabetic patients, 17 men and 29 women with a mean age of 73.2 (32-92) years and 53 diabetic patients (26 men and 27 women), mean age 69 (47-89) years. Ankle pressure was determined in all patients by means of photoplethysmography as a pulse detector.

Statistics

All data is expressed as the mean \pm 1SEM and was analysed by the Student's t-test for paired and unpaired samples. To assess reproducibility of the method the Spearman correlation coefficient and the standard deviation of the difference (Nielsen, Bell and Lassen, 1973) were determined. Analysis of covariance was used to analyse the relationship between SPP and ankle pressure.

RESULTS

MAP, SPP and ankle pressure

The MAP, SPP and ankle pressure for groups I, II and III are shown in fig. 4.1. The MAP in group I was 90 ± 2 mmHg. This was lower than group II (100 ± 2 mmHg; $p < .01$) and group III (101 ± 2 mmHg; $p < .001$). There was no difference between groups II and III.

The SPP in group I was 93 ± 2 mmHg. This was lower than group II (100 ± 2 mmHg; $p < .05$) and higher than group III (82 ± 4 mmHg; $p < .02$). Group II had a higher SPP than group III ($p < .01$). The MAP and the SPP were similar in both groups I and II ($p > .20$ for both groups) but the MAP was higher than the SPP in group III ($p < .0001$).

The ankle pressure in group I (130 ± 3 mmHg) was slightly higher than the systolic pressure (125 ± 3 mmHg; $p < .05$). In group II the ankle pressure (137 ± 4 mmHg) was similar to the systolic blood pressure (137 ± 4 mmHg; $p > .20$). In group III the ankle pressure (105 ± 10 mmHg) was

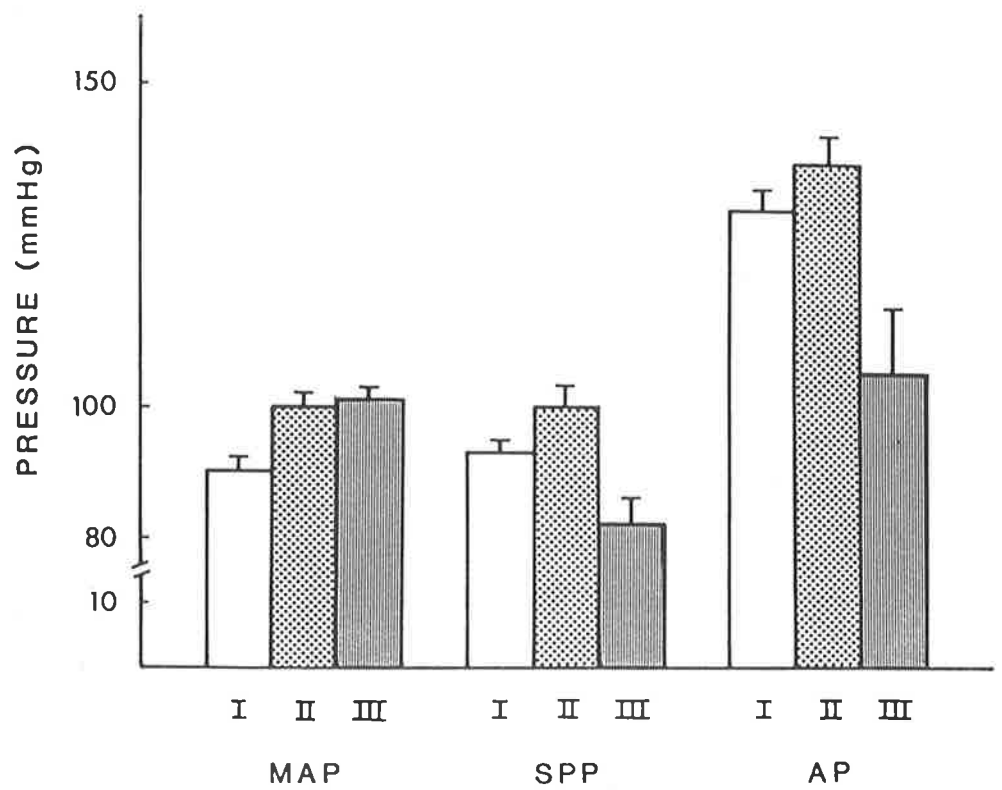


FIGURE 4.1

The mean arterial pressure (MAP), skin perfusion pressure (SPP) and ankle pressure (AP) for groups I, II and III (see text for definition of groups). The values represent the mean + 1 SEM.

lower than the systolic pressure (143 ± 4 mmHg; $p < .01$).

SVR

The results for the SVR between the three groups are shown in fig. 4.2. The SVR in group I (739 ± 28 units) was lower than both group II (1040 ± 57 units; $p < .001$) and group III (995 ± 45 units; $p < .001$). There was no difference between groups II and III ($p > .20$).

kmax

The results for kmax for the three groups are shown in fig. 4.3. kmax for group I ($0.130 \pm .005 \text{ min}^{-1}$) was higher than group II ($0.095 \pm .005 \text{ min}^{-1}$; $p < .001$). kmax in group III ($0.084 \pm 0.006 \text{ min}^{-1}$) was lower than group II ($p > .10$).

SVR vs EVR

The relationship between the SVR and EVR is shown in fig. 4.4. The EVR for group I was 718 ± 37 units and group II 1021 ± 47 units. From the figure it can be seen that

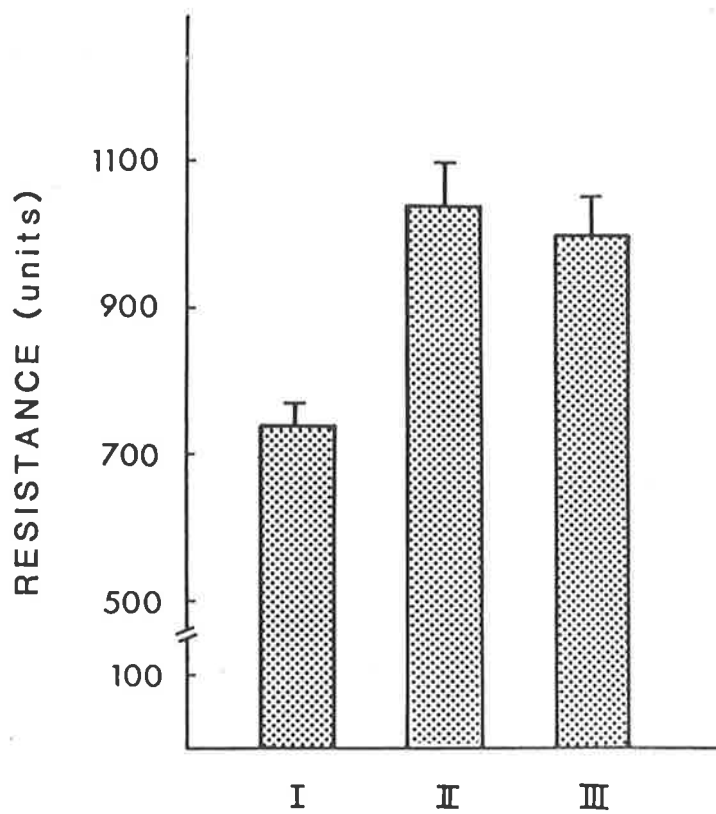


FIGURE 4.2

The skin vascular resistance (mean + 1 SEM) for groups I, II and III.

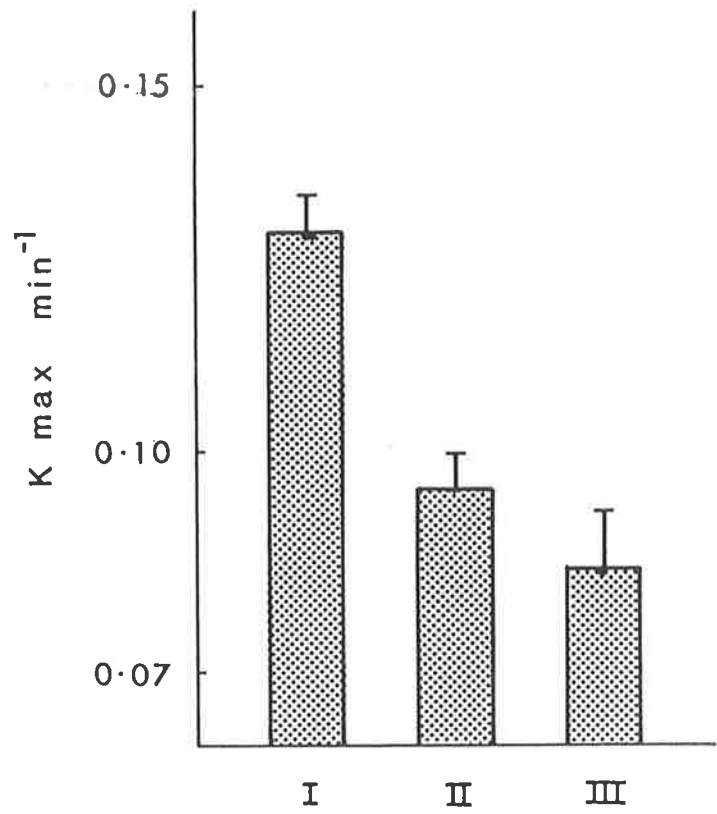


FIGURE 4.3

The washout rates (mean + 1 SEM) for groups I, II and III.

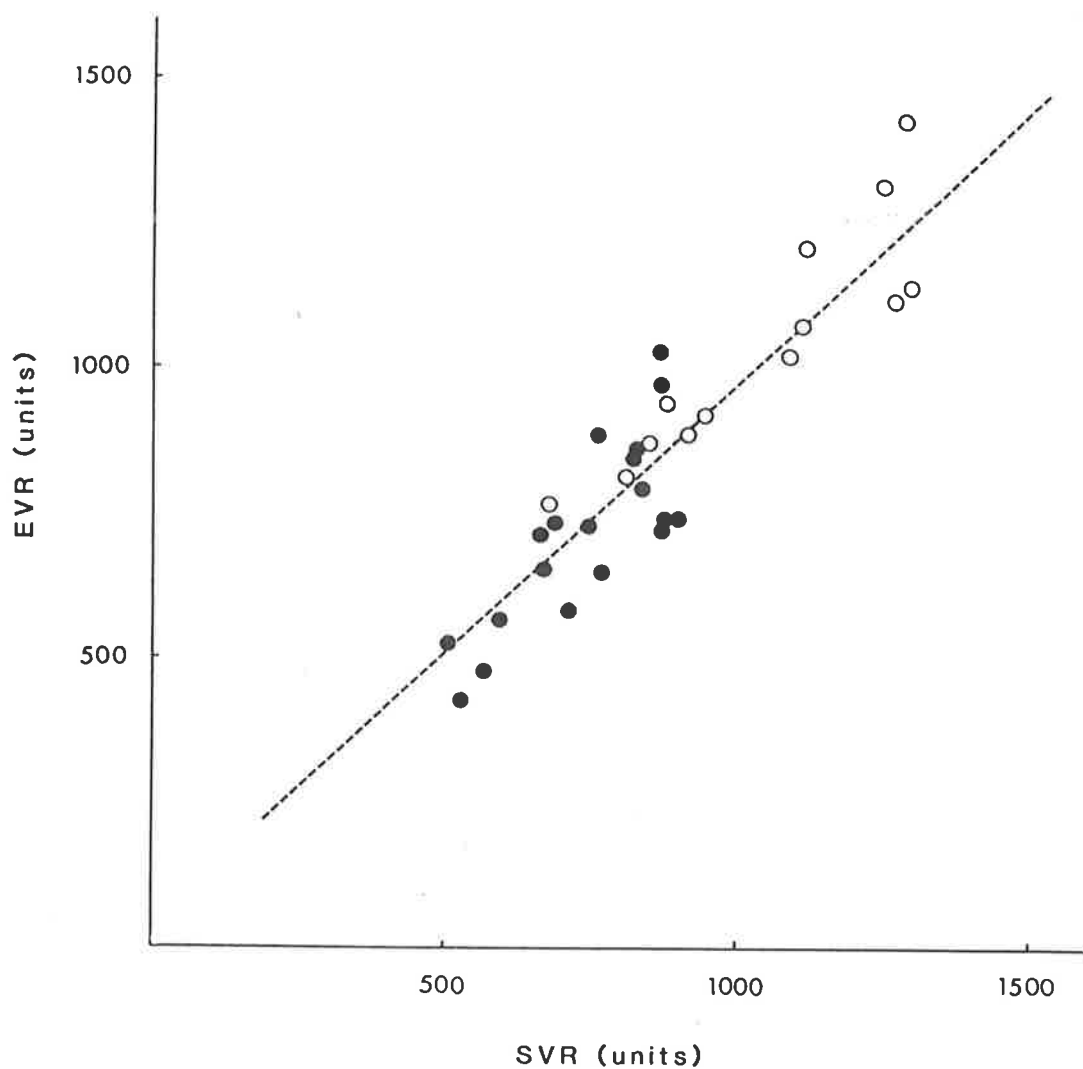


FIGURE 4.4

Comparison between the estimated vascular resistance (EVR) and skin vascular resistance (SVR) for groups 1 (closed circles) and 2 (open circles). The broken line ($y = 0.92x + 43$, $r = 0.88$) is the line of best fit.

there is a very good correlation between SVR and EVR for both groups ($r_s = 0.88$; $p < .0001$).

Reproducibility

The reproducibility of the test is shown in fig. 4.5. The SPP on day 1 was 75 ± 8 mmHg and on day 2 was 70 ± 6 mmHg. The standard deviation of the difference between the two days was 6.5 mmHg and the correlation co-efficient (r_s) was 0.86. The SVR on day 1 was 1285 ± 115 units compared to 1254 ± 109 units on day 2. The standard deviation of the difference was 61 units and the r_s was 0.81. The kmax on day 1 was $0.060 \pm 0.006 \text{ min}^{-1}$ and on day 2 was $0.058 \pm 0.005 \text{ min}^{-1}$. The standard deviation of the difference was 0.004 and r_s was 0.55.

Location

The influence of location is shown in fig. 4.6. The SVR was higher in the calf (1082 ± 70 units) than the foot (870 ± 65 units; $p < .05$). Similarly the SPP was higher in the calf (86 ± 6 mmHg) when compared to the foot (69 ± 7

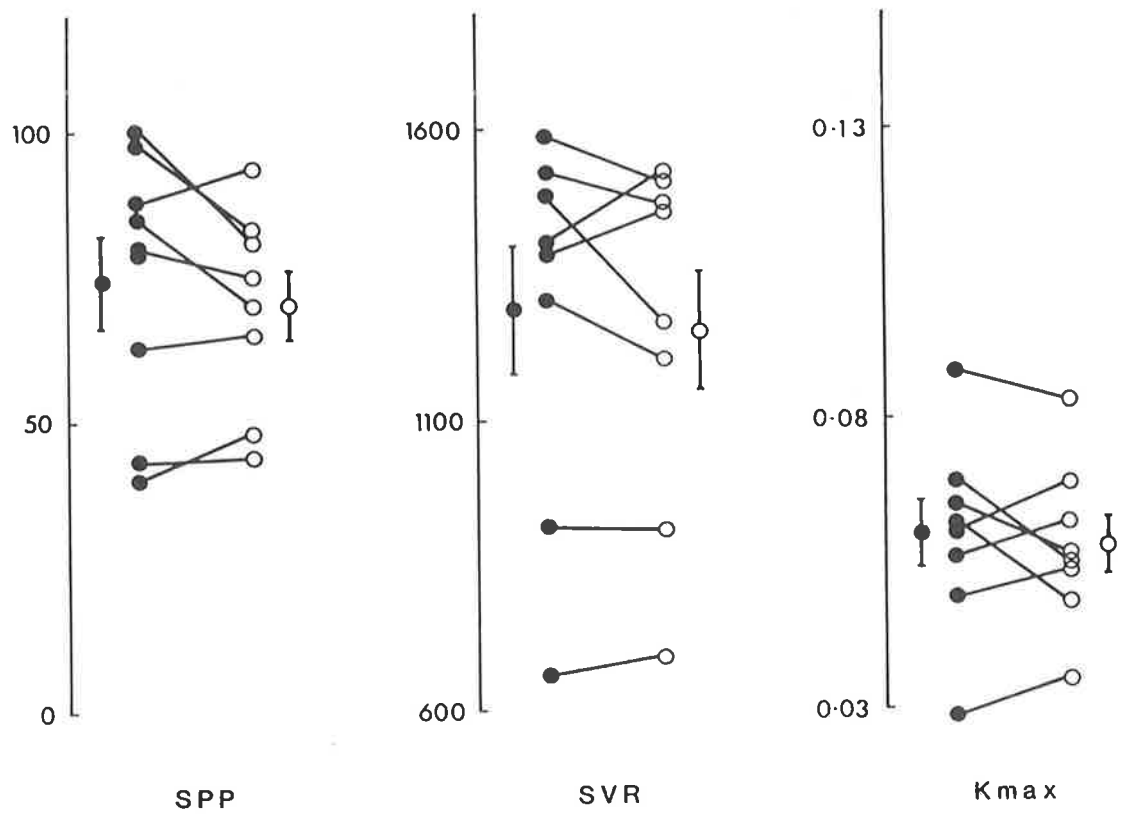


FIGURE 4.5

Reproducibility of SPP, SVR and kmax. The closed circles represent day 1 and the open circles day 2.

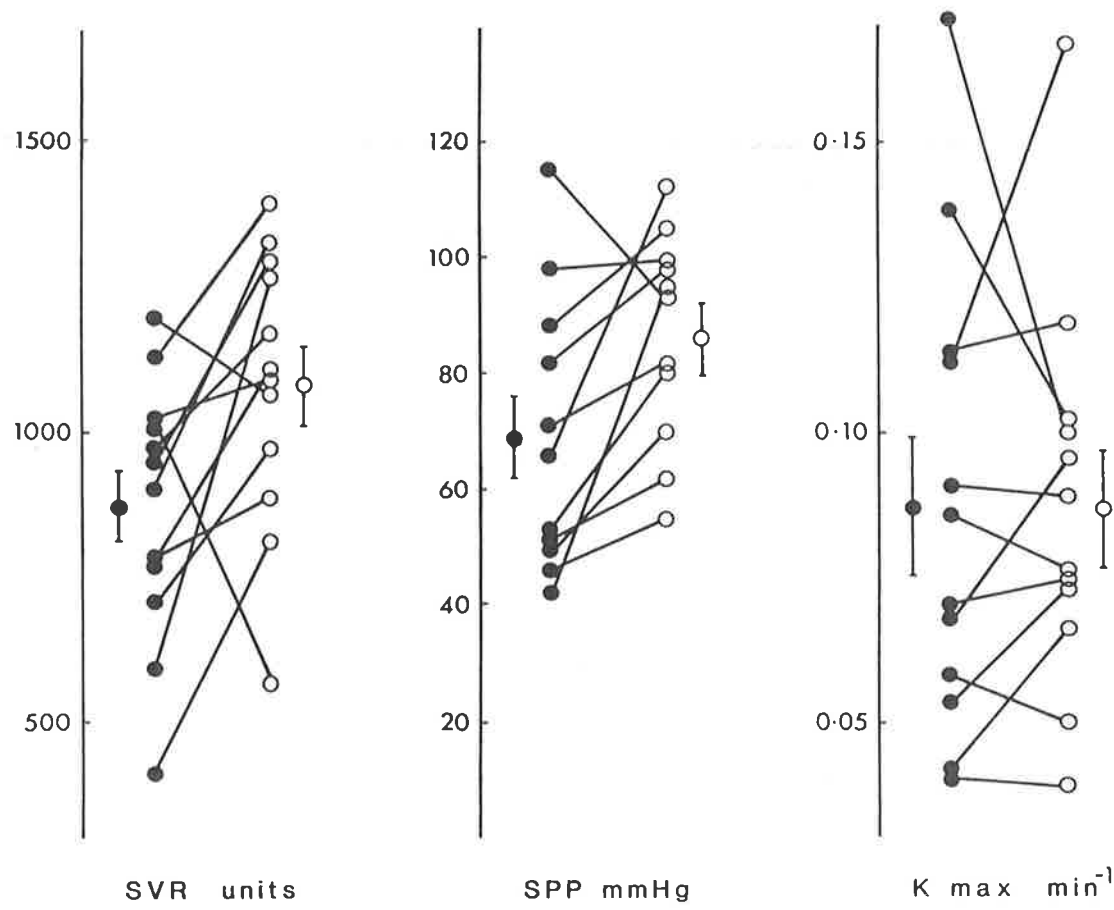


FIGURE 4.6

Influences of location on SVR, SPP and kmax. The closed circles represent the foot and open circles the calf.

mmHg; $p < .01$). The k_{max} was similar in the calf ($0.087 \pm .01 \text{ min}^{-1}$) and the foot ($0.087 \pm .01 \text{ min}^{-1}$; $p > .20$).

Surgery

The effect of surgery is shown in fig. 4.7. The post-operative SVR (907 ± 113 units) was lower than the pre-operative value (1145 ± 73 units; $p > .20$). The post-operative SPP (77 ± 7 mmHg) was higher than pre-operatively (37 ± 9 mmHg; $p < .01$) as was the k_{max} ($0.096 \pm .015 \text{ min}^{-1}$ compared to $0.033 \pm .01 \text{ min}^{-1}$; $p < .01$).

Ankle pressure vs SPP

The ankle pressure, MAP and SPP in the nondiabetic and diabetic groups are shown in fig. 4.8. The MAP was 103 ± 1.5 mmHg in the nondiabetic group and 106 ± 1.6 mmHg in the diabetic group ($p > .10$). The SPP was 72 ± 4 mmHg in the nondiabetic group and 63 ± 3 mmHg in the diabetic group ($p > .10$). The ankle pressure was lower in the nondiabetic group (104 ± 6 mmHg) compared to the diabetic group (127 ± 8 mmHg; $p < .05$). The relationship between the SPP and the

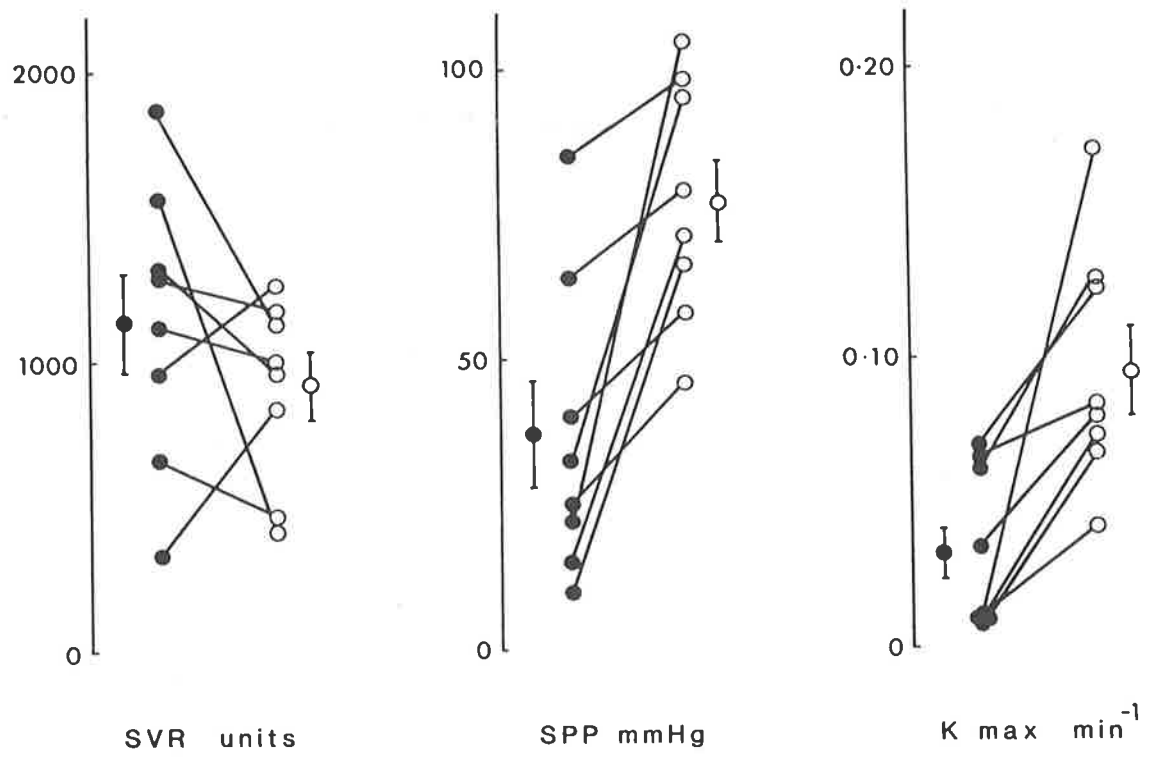


FIGURE 4.7

SVR, SPP and kmax before (closed circles) and after (open circles) femoropopliteal surgery.

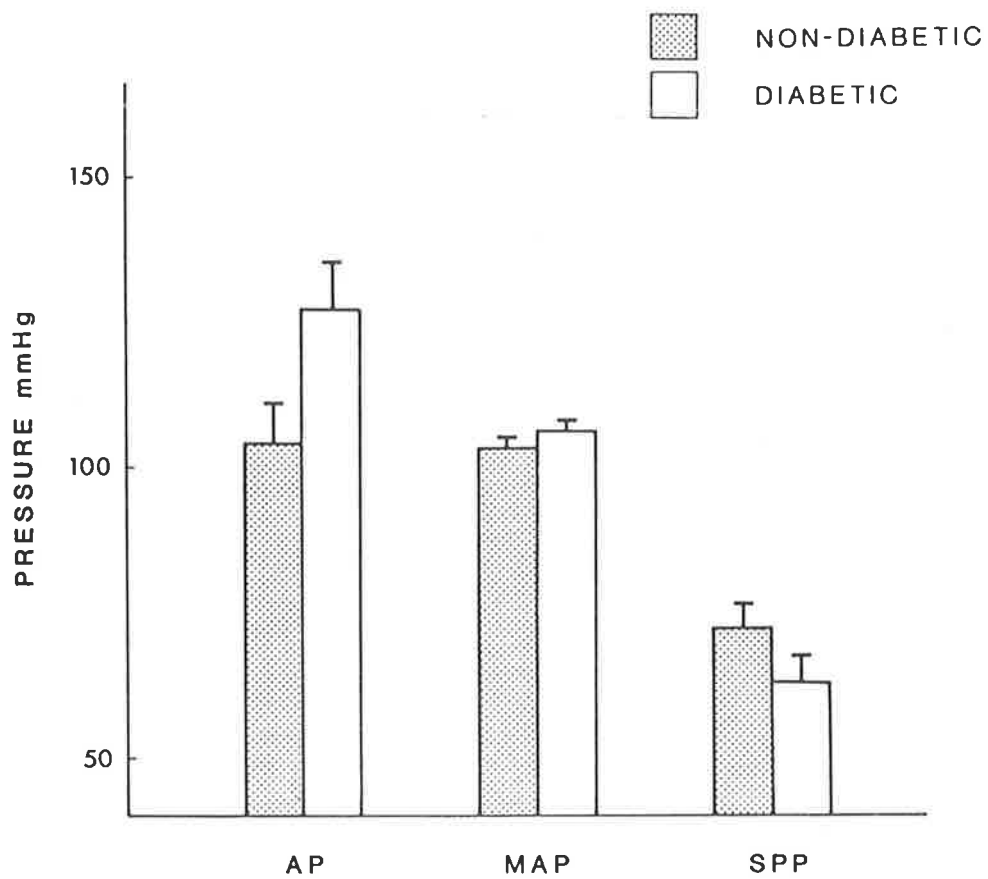


FIGURE 4.8

The mean (+ 1 SEM) ankle pressure (AP), MAP, and SPP in the non-diabetic and diabetic groups.

ankle pressure is shown in fig. 4.9. In the diabetic group the relationship is $y = 0.20 x + 37$ ($r = 0.34$; $p < .05$) and for the nondiabetic group is $y = 0.41x + 30$ ($r = 0.50$; $p < .001$). When analysed by analysis of co-variance the two groups were different ($F = 8.07$; $df = 1.98$; $p < .001$). This indicates that in the diabetic group the arteries tend to be less compressible resulting in higher ankle pressure recordings.

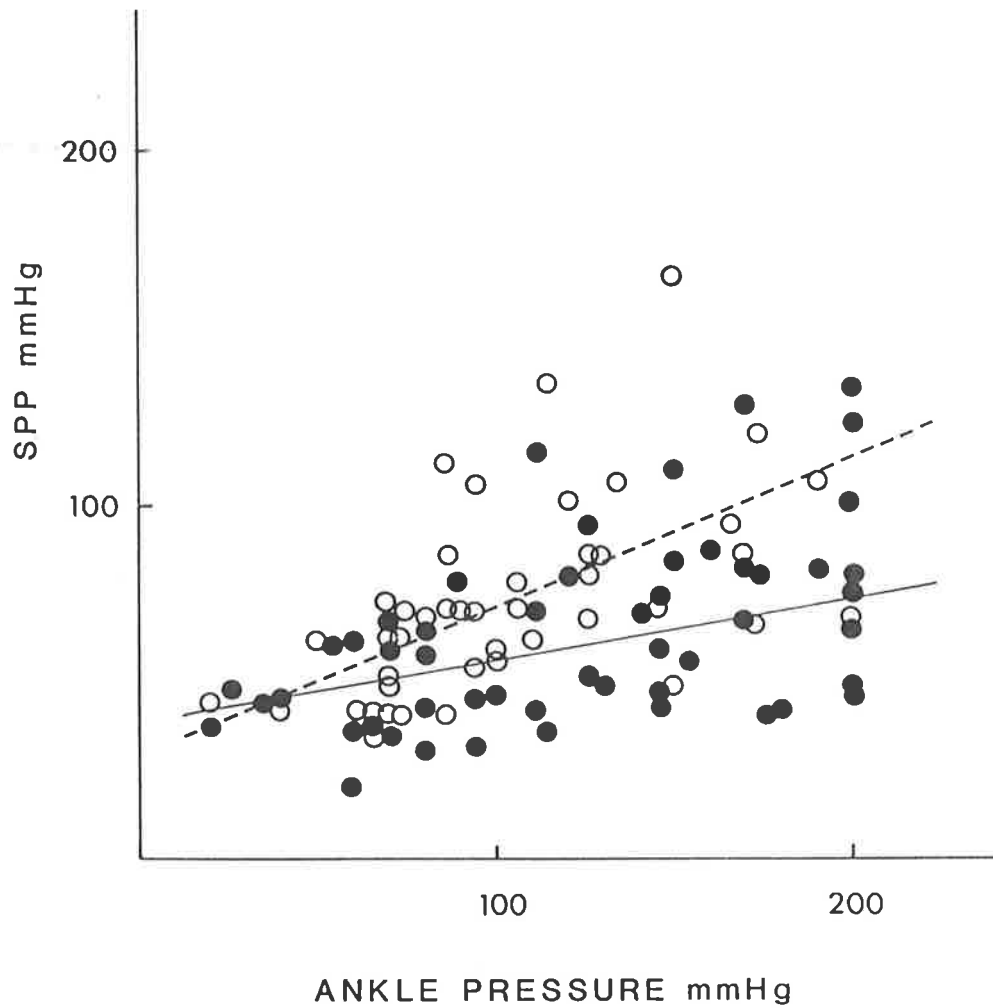


FIGURE 4.9

Relationship between SPP and ankle pressure in the diabetic (closed circles) and non-diabetic groups (open circles). The dotted line is the line of best fit for the non-diabetics and the solid line for the diabetics. See text for details.

DISCUSSION

The SPP has been calculated in subjects with and without peripheral vascular disease (PVD). In patients without PVD the SPP is higher in the older age group compared to the younger group as was MAP and the ankle pressure. The SPP was equivalent to the MAP in both these groups. Holstein et al (1977; 1982) studied the relationship between the SPP and directly recorded femoral artery pressure and found similar results. They concluded that the SPP was an indirect measure of the wedge pressure, that is, the pressure in the main artery supplying the tissue being studied. Thus the SPP appears to be useful in assessing the severity of PVD. This was confirmed in nondiabetic patients where there was a significant correlation between the ankle pressure and the SPP in patients with PVD (fig. 4.9). In diabetic patients there was poorer correlation. The most likely explanation is the presence of arterial calcification seen in diabetic patients (Ferrier, 1964; Neubauer, 1971) which reduces the compressibility of the arteries thus making the estimations of the ankle pressure less reliable as an indicator of

intravascular pressure (Hanser et al, 1984). There is little data on the magnitude of this problem. Carter (1973) found that in four out of five limbs with extensive calcification, the indirectly measured pressure was greater than 10 mmHg higher than direct pressure recordings. Gibbons et al (1979) found indirectly measured pressures of greater than 200 mmHg in 22 of 150 patients. In our study 8 out of 53 diabetic patients had ankle pressures greater than 200 mmHg. However, of particular interest was the poor correlation between the SPP and ankle pressure for all ranges of ankle pressures in diabetic patients. The SPP tended to be much lower than the ankle pressure in the diabetic groups compared with the nondiabetic group suggesting that the interpretation of ankle pressures in diabetic patients is difficult for all ranges of ankle pressure. For this reason, the SPP may be of particular value in the assessment of PVD in diabetic patients.

The conventional method of calculating resistance in the vascular bed is to divide the perfusion pressure by the blood flow. Skagen and Henriksen (1983) by assuming that the perfusion pressure in the lower limb is equivalent to

the MAP in a supine patient, used this method to estimate the vascular resistance in the subcutaneous tissue. They used the washout rate (k) to represent blood flow. The SVR measured by the method described in chapter 2 was equal to the vascular resistance estimated (EVR) by the method described by Skagen and Henriksen (1983) in subjects without PVD. This important observation validates the method used in this thesis to measure SVR. In patients with PVD the MAP in the distal arterial tree is not known and, therefore, the resistance can only be calculated by the technique used in this thesis.

We have shown in this study that the SVR increases with age, is unaffected by atherosclerosis and varies with different anatomical locations. The increase with age supports histological studies which have demonstrated thickening of the arteriolar wall with increasing age (Bone and Pomajzl, 1981; Fishberg, 1925). These age-related changes significantly alter the physiology of the microcirculation. There is a reduced reactive hyperaemia in the human calf (Kriese, 1977) and a decrease in arterial distensibility (Smulyan et al, 1983). Despite a slightly

higher SPP with age the elevated SVR reduced k_{max} by approximately 27% (fig 4.3) reflecting a corresponding decrease in skin blood flow.

An interesting finding of this study is the observation that atherosclerosis of the leg arteries does not appear to involve the microvessels as the SVR was not altered in these patients. It would be expected that if the microvessels were involved there would be narrowing of the vessels increasing the resistance. Therefore, the age-related arteriosclerosis appears to have a different pathogenesis to atherosclerosis.

There were regional variations in the SPP and SVR as both these parameters were 25% higher in the calf compared to the foot. This variation in SPP has been reported before where it has been found that there is 12-48% reduction in the SPP in the foot compared to the calf (Holstein et al, 1977; 1983). These workers also measured the SPP at thigh and ankle level and found no difference to the calf. This is logical as the blood pressure is reduced in the distal arterial tree especially in the region of the medium sized

metatarsal arteries as indicated by toe blood pressure estimations. The reduced SVR in the foot could be an adaptation to the lower SPP so that the skin blood flow is maintained within normal limits. Indeed, the kmax were identical in the foot and calf.

Arterial reconstructive surgery resulted in a 110% increase in the SPP and 190% increase in the kmax (and therefore blood flow) in the foot. The kmax increased to a greater degree than the SPP and this can be explained by a 20% reduction in the SVR, a fall which did not reach statistical significance, although the numbers studied were small. It is possible that the SVR decreases in these patients post-operatively because there is an increased distal blood pressure which distends the arterioles, increasing their diameter and thus decreasing their resistance.

This study indicated that the test is reproducible with small standard deviations of the differences between recordings on adjacent days. In particular the SVR was within 10% of its original estimation in all patients. The

SPP and kmax tended to have a larger variation but this is not surprising as there tends to be slight day to day variations in systemic blood pressure.

In conclusion this study has demonstrated that this technique to measure SPP and SVR is reliable and reproducible. The SPP appears to be an indicator of the severity of PVD and is of particular value in diabetic patients in whom the ankle pressure estimation is unreliable because of arterial calcification. The SPP and SVR are influenced by age and location and, therefore, these parameters need to be taken into consideration when interpreting the results. The effect of arterial surgery has also been demonstrated and this test may be of value in assessing the effectiveness of such surgery.

CHAPTER 5

SKIN VASCULAR RESISTANCE IN DIABETES MELLITUS AND HYPERTENSION.

INTRODUCTION:

In the previous chapter the measurement of SVR and SPP in normal subjects and in patients with PVD was described. The SPP is a measure of the wedge pressure of the main arteries of the leg and thus appears to be useful in the estimation of severity of atherosclerotic arterial disease. In this chapter we will examine the hypothesis that the SVR provides information concerning the structure of the skin microvessels and that elevation of the SVR indicates structural abnormalities of these vessels by studying patients with diabetes mellitus and hypertension.

Initial studies in heterogeneous groups of patients confirmed that the SVR was elevated in some diabetic and hypertensive patients and these findings will be discussed in the first part of this chapter. As a consequence of

these initial findings more homogeneous groups were studied and will be discussed in the second part of the chapter.

STUDIES IN HETEROGENEOUS GROUPS

METHODS:

Subjects:

This groups consisted of 122 patients in whom 127 studies were performed. The patients were divided into four subgroups depending on whether they were nondiabetic (D-H-), diabetic nonhypertensive (D+H-), nondiabetic hypertensive (D-H+) or diabetic hypertensive (D+H+). Hypertension was considered present if the patient was regularly receiving anti-hypertensive therapy. Similarly diabetes mellitus was considered present if the patient was receiving regular therapy, such as insulin, oral hypoglycaemic agents or diet, to regulate blood glucose which was elevated on repeated fasting or 2 post-prandial blood glucose measurements. Patient characteristics are shown in table 5.1. Of these 122 patients 102 were referred to the vascular laboratory for investigation of lower limb ischaemia. The remaining 20

TABLE 5.1 - PATIENT CHARACTERISTICS OF HETEROGENEOUS GROUPS

				DURATION OF	
	NUMBER	MALE	AGE (years)	DIABETES (years)	HYPERTENSION (years)
D-H+	45	17	68 (34-85)	-	-
D-H+	14	3	68 (50-84)	-	10 (2-30)
D+H-	42	29	69.5 (38-88)	17 (2-30)	-
D+H+	21	12	64 (40-82)	6.5 (0.5-40)	10 (3-30)

patients had multiple sclerosis and were having unrelated studies performed in our laboratory. The SVR was measured in all patients by the method described in chapter 2. Histamine (50 micro g) was used to paralyse the vascular bed and the SVR was estimated on the dorsum of the foot or the lower calf if the foot was not suitable for study.

Histological Studies:

Operative specimens were taken from ten patients who underwent either digital amputation (n=5) or below knee amputation (N=7) for treatment of severe limb ischaemia. Histological preparation and study was performed by a histopathologist (Dr. Amanda Gramp) who had no knowledge of the patient's clinical history or SVR.

Specimens were fixed at 10% formal saline at the time of the operation and processed into paraffin and sectioned at 5 micro m. Sections were stained using haematoxylin and eosin, periodic acid schiff (PAS) and Miller's stain for elastic counter stained with Van Gieson.

Assessment was made of:

- (i) small arteries with reference to intimal and medial thickening,
- (ii) arterioles with reference to arteriosclerosis, endothelial proliferation and basement membrane thickening,
- (iii) capillaries with reference to endothelial proliferation and basement membrane thickening.

The changes were graded on an arbitrary scale of

0 = no significant changes,

1 = mild,

2 = moderate, and

3 = severe changes.

Statistics:

Results were expressed as median and range and were analysed by the non-parametric Wilcoxon test for unpaired observations.

RESULTS

SVR:

The results are shown in fig. 5.1. The SVR in the D-H- group was 688 (255-1084) units. In the D-H+ group the SVR was 1045 (463-1796) units which is higher than the D-H- group $p < .001$). The SVR was also elevated in the D+H- group (854; 354-1830 units; $p < .01$) and the D+H+ group (885; 554-2652 units; $p < .001$) when compared to the D-H- group.

Histology

The histology results are shown in table 5.2. None of the biopsies revealed arteriolar or capillary endothelial proliferation. Biopsies from two patients (nos. 2 and 3) showed moderate arteriolar and capillary basement membrane thickening in the absence of diabetes. In addition, the biopsy from patient no. 9 showed only minimal arteriolar and capillary basement membrane thickening. However, this patient had severe changes to the small arteries.

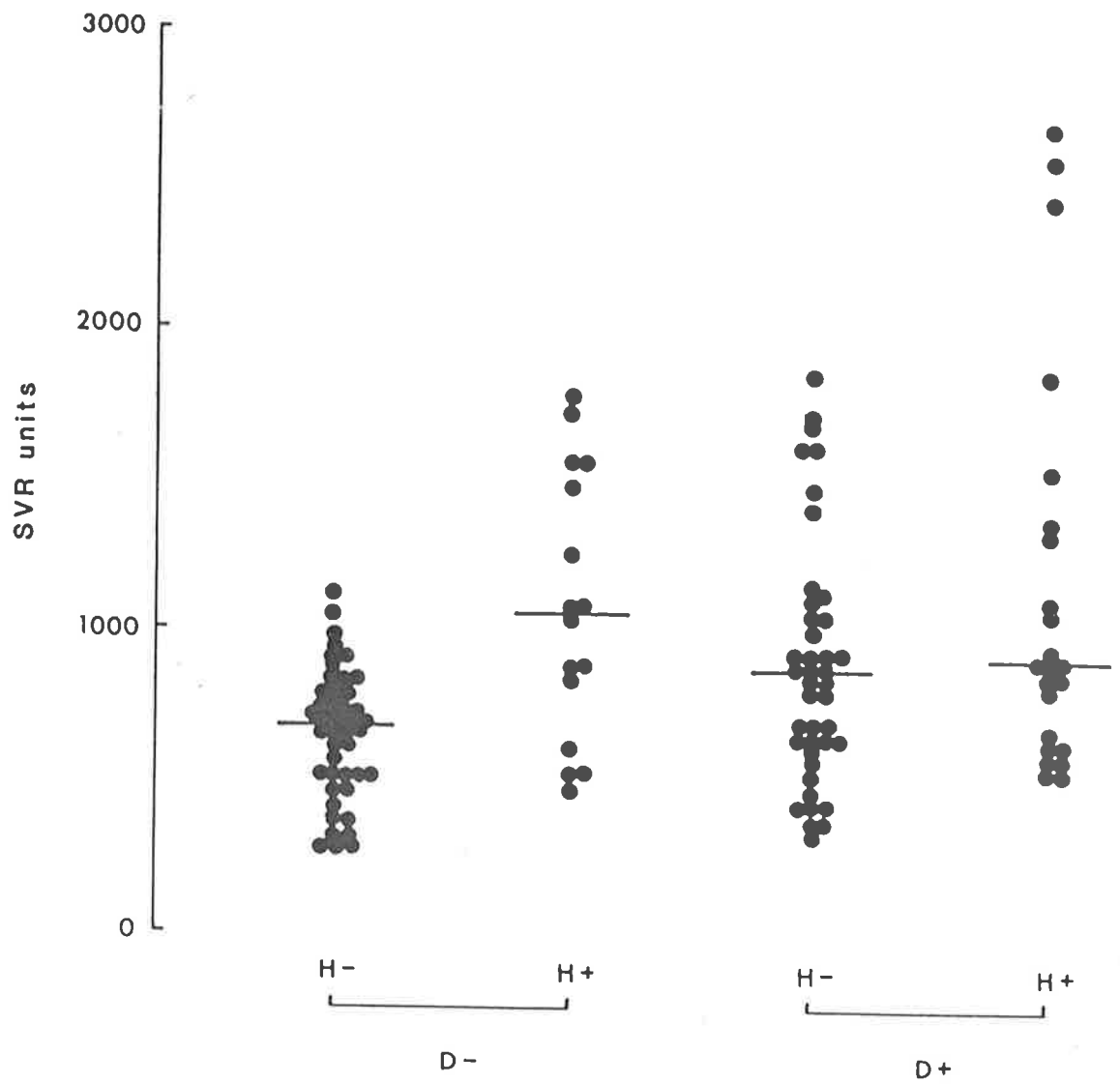


FIGURE 5.1

SVR in the non-diabetic non-hypertensive (D-H-), non-diabetic hypertensive (D-H+), diabetic non-hypertensive (D+H-) and the diabetic hypertensive (D+H+) groups.

TABLE 5.2 - HISTOLOGICAL ASSESSMENT

Patient No	Arteries Intima	Arteries Media	Arterioles BM Thickening	Arterioles Arteriolo-sclerosis	Capillaries BM Thickening	Diabetic/Hypertensive Status	SVR
1.	2	2	0	1	0	D- H-	747
2.	3	2	3	2	2	D- H-	1662
3.	2	2	2	0	2	D- H+	1342
4.	2	2	0	1	1	D+ H-	658
5.	1	1	1	0	2	D+ H-	700
6.	1	3	2	0	2	D+ H-	1186
7.	2	2	2	3	2	D+ H+	1100
8.	2	2	3	3	3	D+ H+	1600
9.	2	3	1	0	1	D+ H+	1516
10.	1	2	2	1	3	D+ H+	1121

Comparison between the histological grading and SVR demonstrated that the SVR is higher with increasing histological grades.

DISCUSSION

The structural changes to the skin microvessels have been discussed in chapter 1. In diabetes these changes include:

- (i) basement membrane thickening most obvious in the capillaries,
- (ii) endothelial or intimal proliferation, and
- (iii) hyaline arteriosclerosis (Goldenberg et al, 1959).

In hypertension the changes are:

- (i) intimal hyalinisation,
- (ii) intimal proliferation,
- (iii) media hypertrophy and degeneration (Moritz and Oldt, 1937).

The physiological consequences and the lack of a specific diagnostic test to detect these changes were also discussed in detail in chapter 1.

The aim of this present work was to examine the hypothesis that elevation of the skin vascular resistance specifically indicates structural changes to the

microvessels. This is based on the assumption that the changes described above might result in changes to the cross sectional dimensions of the vessel wall. Folkow, Grimsby and Thulesius (1958) introduced the concept of the wall/lumen ratio with a thickening of the wall and a narrowing of the lumen in hypertension. The changes seen in diabetes could result in similar changes with the wall/lumen ratio increasing. From the Poiseuille formula the narrowing of the lumen would be expected to increase the resistance to blood flow through the vessel. Therefore, measurement of the SVR may provide a method for detection of structural alterations of the skin microvessels. This hypothesis relies upon the assumption that the vessels under study were maximally dilated, that is, the vascular smooth muscle is paralysed and unresponsive to nervous and humoral controls. If this was not the case then alterations in muscle tone would interfere with the SVR measurements. It is for this reason that the vessels were paralysed with histamine or nitroprusside.

In this study the SVR was found to be elevated in a large number of diabetic and hypertensive patients with

lower limb ischaemia. This study consisted of heterogeneous groups in that some patients were recently diagnosed diabetics and hypertensive while others were long standing. Apart from duration other variables not taken into consideration include severity, control and complications of these conditions. Because of the heterogeneity of these groups it is difficult to establish firm conclusions about the SVR and the presence of microvascular disease.

The histological studies were performed to compare directly the SVR with the presence of microvascular disease. These studies are necessary as it is theoretically possible for other factors to influence the SVR. For example, differing sensitivities of the vessels to the vasodilatory agent in different disease states would influence the SVR. Another important variable is the vessel density in different disease states. Hutchins and Dornell (1974) and Gray (1984) have demonstrated reduced vessel density in hypertensive rats compared with normo-tensive rats. A reduced vessel density would be expected to increase vascular resistance of the tissue under study. Unfortunately histological specimens were obtained from only

10 patients (table 5.2). Despite these small numbers the SVR does appear to reflect microvascular disease. Further histological studies are essential to determine the specificity and sensitivity of the SVR to detect microvascular disease.



STUDIES IN HOMOGENEOUS GROUPS:

Methods:

To further investigate the relationship between SVR and microvascular disease studies were performed on homogeneous groups of diabetic and hypertensive patients. The patients studied consisted of 13 non-diabetic non-hypertensive subjects, 15 subjects with a long standing history of hypertension, 9 subjects with minimal retinopathy secondary to insulin dependent diabetes of greater than five years duration, and 13 subjects with severe proliferative diabetic retinopathy requiring laser therapy. All patients were free from peripheral vascular disease. Patient details are summarised in table 5.3.

The SVR, SPP and kmax were measured in all patients as described in chapter 2. Nitroprusside (250 micro g) was used and the area of study was the anteromedial aspect of the calf midpoint between the malleolus and the patella.

TABLE 5.3 - PATIENT CHARACTERISTICS OF HOMOGENEOUS GROUPS

	Number	Male	Age (range)	Diabetes	Hypertension
CONTROL	13	6	59 (33-86)	-	-
HYPERTENSION	15	7	62.5 (39-89)	-	15 (7-34)
MILD					
RETINOPATHY	9	4	60 (20-82)	10 (5-20)	-
SEVERE					
RETINOPATHY	13	6	61 (27-74)	10 (.25-44)	-

RESULTS

The results of the SVR are shown in fig. 5.2. The SVR in the control group was 1087 (684-1304) units which was similar to the minimal retinopathy group (957; 571-1473 units; $p > .10$). However, the SVR was higher in the diabetic group with severe retinopathy (1440; 766-2048 units; $p < .001$) and the hypertensive group (1478; 1077-2321 units; $p < .001$) compared to the control group.

The SPP results are shown in fig. 5.3. The SPP for the control group 100 (83-119)mmHg was similar to the minimal retinopathy group (97; 79-110mmHg, the severe retinopathy group (112; 56-122mmHg) and the hypertensive group (116; 56-161mmHg).

The kmax results are shown in fig. 5.4. The kmax for the control group (0.98; 0.076-0.126 min⁻¹) was similar to the minimal retinopathy group (0.11; 0.067-0.147; $p > .01$). The kmax in the severe retinopathy group (0.071; 0.055-0.111 min⁻¹) and the hypertensive group (0.071; 0.033-0.108 min⁻¹) were lower than the control group ($p < .01$).

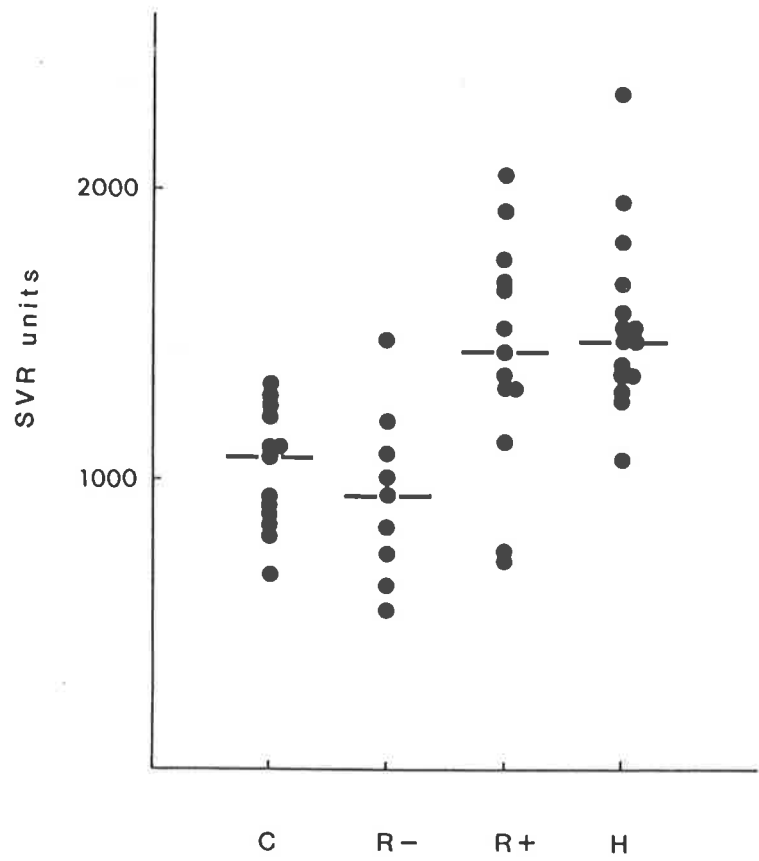


FIGURE 5.2

SVR in control (C), minimal retinopathy (R-), severe retinopathy (R+) and hypertensive (H) groups.

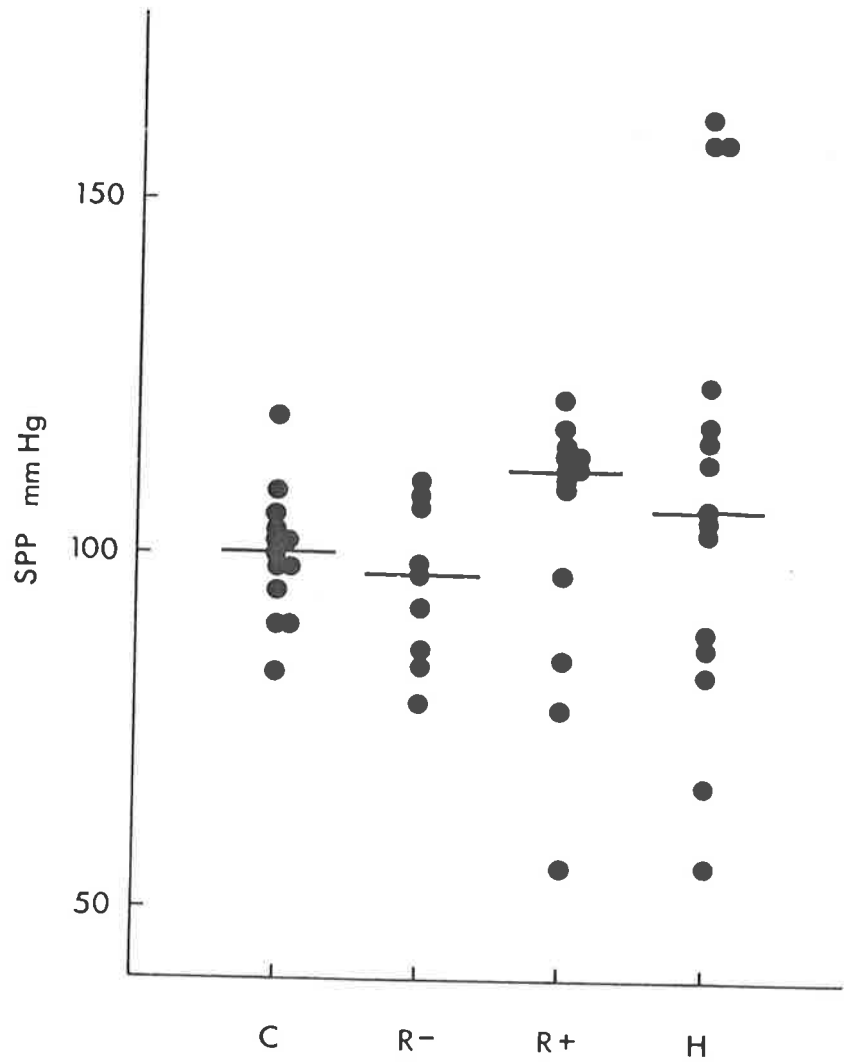


FIGURE 5.3

SPP in the control (C), minimal retinopathy (R-), severe retinopathy (R+) and hypertensive (H) groups.

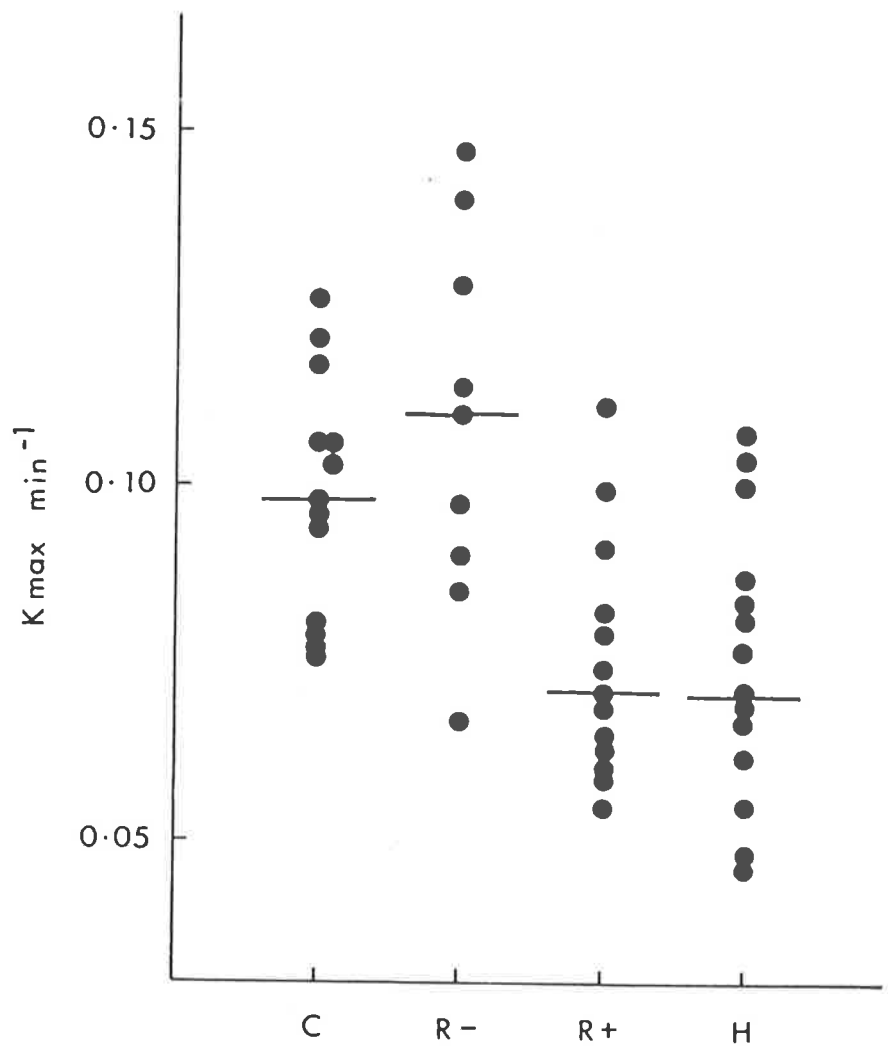


FIGURE 5.4

SVR in the control (C), minimal retinopathy (R-), severe retinopathy (R+) and hypertensive (H) groups.

DISCUSSION

The results of this study further support the hypothesis that an elevated SVR reflects microvascular disease. In diabetic patients with severe retinopathy (R+) requiring laser therapy, the SVR was elevated compared to the control group (C) and the diabetic group with minimal retinopathy (R-). Although the relationship between skin microvascular disease and retinopathy is not known it is assumed that skin microvascular disease is more likely to be present in patients with severe retinopathy. This assumption is based on the finding that renal microvascular changes evolve in parallel with retino-vascular changes (Paz-Guevara, Hsu and White, 1975; Kussman, Goldstein and Gleason, 1976). Therefore, in patients in whom there was a high likelihood of skin microvascular disease the SVR was elevated compared to the diabetic patients in whom the likelihood of skin microvascular disease is low. In patients with long standing hypertension widespread microvascular changes are expected and this is supported by the finding of increased SVR in this group.

Three of the patients in this study had evidence of significant peripheral vascular disease with a SPP below normal range. In patients without peripheral vascular disease the skin blood flow (indicated by $k_{max} \text{ min}^{-1}$) was reduced in the hypertensive and R+ group indicating that even in the absence of peripheral vascular disease microvascular disease alters blood flow. This is an important observation as it may explain why healing of lower limb ischaemic ulceration and gangrene is reduced in these patients (see chapter 6).

In this chapter an attempt was made to correlate abnormal SVR with the presence of skin microvascular disease. Although an absolute correlation was not possible because of the small number of histological studies performed the finding of an elevated SVR in diabetic patients with severe retinopathy and patients with long standing hypertension support the correlation. Of particular interest was the finding that in patients with an elevated SVR there was a corresponding decrease in skin blood flow even in the absence of PVD. This will be discussed in the following chapter.

CHAPTER 6

SKIN PERFUSION PRESSURE AND SKIN VASCULAR RESISTANCE IN THE MANAGEMENT OF LOWER LIMB ISCHAEMIA

INTRODUCTION

The assessment of skin blood flow is necessary when managing a patient with lower limb ischaemia. Although clinical observation of an ischaemic lesion over a period of time is the most common method for determining the adequacy of tissue blood flow, this approach often results in prolonged unsuccessful trials of conservative therapy or failed local amputation resulting in further surgery. Numerous techniques have been devised to assess skin blood flow. These techniques and their limitations have been discussed in chapter 1.

In the preceding chapters the measurement of the skin perfusion pressure (SPP) and skin vascular resistance (SVR) were described. It was proposed that the SPP is a measure of

the pressure in the large and medium sized arteries and is a measure of the severity of disease of these arteries, while the SVR is a measure of microvascular disease. The aim of this study was to assess the value of SPP and SVR in the management of lower limb ischaemia and to examine the role of hypertension, diabetes mellitus and age in the pathogenesis of lower limb ischaemia.

METHODS

Subjects

Eighty-seven patients were studied. There were 42 women and 45 men and their median age was 69 (34-88) years. Thirty-one patients were non-diabetic non-hypertensive, 11 were hypertensive, 34 diabetic and 17 had both diabetes and hypertension. The classification of hypertension and diabetes is discussed in chapter 5. Forty-six patients presented with toe ulceration or gangrene, and 41 with foot or leg ulceration.

SVR and SPP were estimated by the method described in chapter 2. In this study histamine was injected with the Tc-99m to paralyse the vascular bed. The injection was placed in normal skin on the dorsum of the foot away from areas of gangrene, infection or ulceration. In 17 patients injections were not possible on the foot and, therefore, were placed on the lower leg as close to the foot as possible.

Patient Outcome

The patient's management was based on clinical features and routine investigations. The patients, who were reviewed at least three months following initial hospital admission, were assessed as having lesions which were 'healed' or 'unhealed'. They were classified as healed if there was successful conservative therapy or healed distal amputation. If the patients required reconstructive arterial surgery, major amputation (above knee or below knee) or if the lesion was not healed at review then they were classified as unhealed.

Statistics

All data ~~are~~ expressed as a median and range and ~~were~~ analysed by the ranksum Wilcoxon test for unpaired samples and the Chi-squared test for independent samples.

RESULTS

Patient Outcome

Forty-four patients were categorised as having lesions which healed. These included 26 patients who had successful conservative therapy and 18 patients who had successful distal amputations. Forty-three patients had lesions which were unhealed by local treatment. Thirty of these had reconstructive arterial surgery with subsequent healing in 21. Eight of these patients had unsuccessful surgery with six requiring major amputation and two having unhealed lesions at review. One patient died following surgery. Seven patients required primary major amputation and 6 had unhealed lesions when reviewed at three months.

Factors affecting healing

(1) Age: The age distribution for the healed and unhealed groups is shown in figure 6.1. The median age for the healed group was 64 (34-85) years and for the unhealed group was 73 (43-88) years ($p < 0.001$).

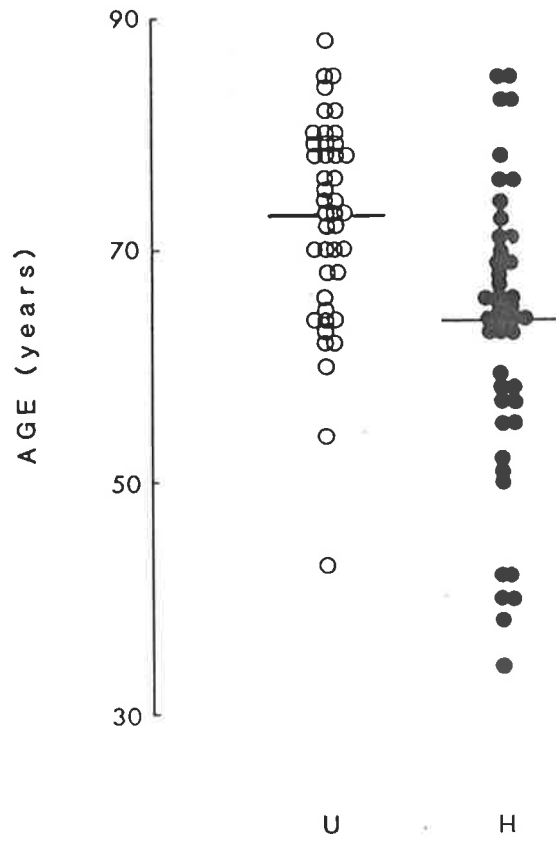


FIGURE 6.1

Age distribution for the unhealed (U) and healed (H) groups.

(2) Diabetes mellitus and hypertension: The influence of diabetes mellitus and hypertension on the healing of lower limb ischaemia is shown in figure 6.2. Of the 25 non-diabetic, non-hypertensive patients 18 (72%) had healed lesions. Four of the 11 (30%) hypertensive patients, 17 of the 34 (50%) diabetic patients and 6 of the 17 (29%) patients who had both hypertension and diabetes had healed lesions. This is significant when analysed by Chi-squared analysis ($\chi^2 = 22.6, p < .001$).

(3) Skin blood flow: The relationship between the maximum washout rate (k_{max}) and healing is shown in figure 6.3. The median k_{max} for the healed group was 0.096 (0.044 - 0.244) min^{-1} and for the unhealed group was .0.043 (0.019 - 0.134) min^{-1} ($p < 0.0001$). Table 6.1 shows the percentage of patients healed for different ranges of k_{max} .

(4) Skin perfusion pressure: The relationship between skin perfusion pressure (SPP) and healing is shown in figure 6.4. The median SPP in the healed group was 60 (30-131) mmHg and in the unhealed group was 43 (27-144) mmHg ($p < 0.0001$). Table 6.1 shows the percentage of patients healed for

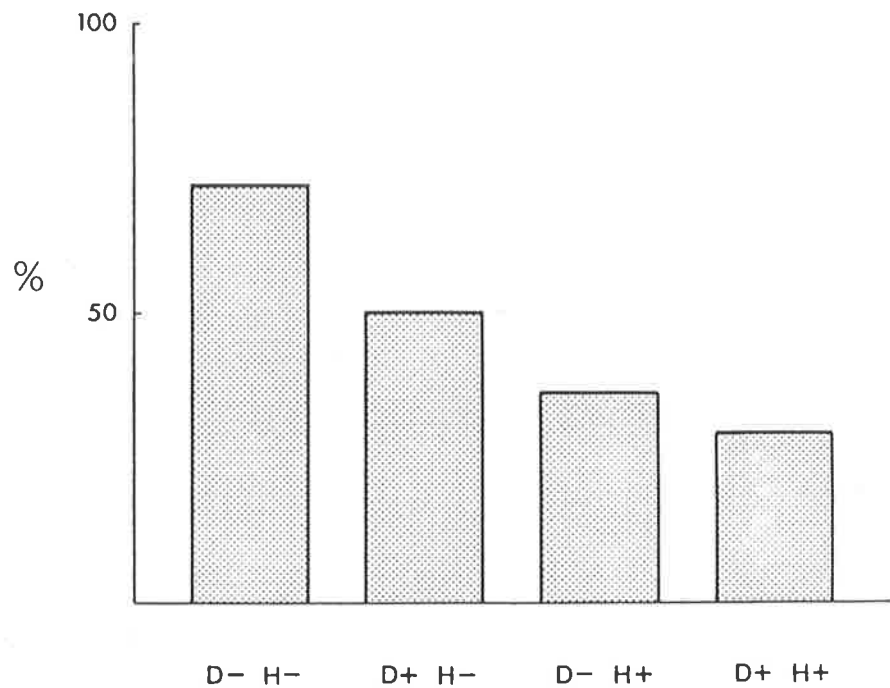


FIGURE 6.2

The percentage healed in the non-diabetic non-hypertensives (D-H-), diabetic non hypertensives (D+H-) non diabetic hypertensives (D-H+) and diabetic hypertensives (D+H+) groups.

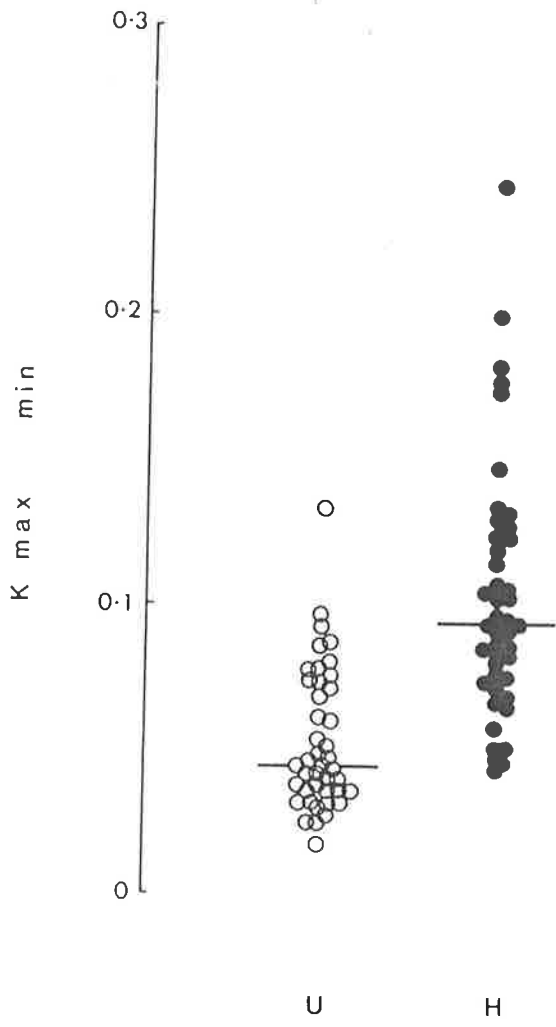


FIGURE 6.3

kmax in the unhealed (U) and healed (H) groups.

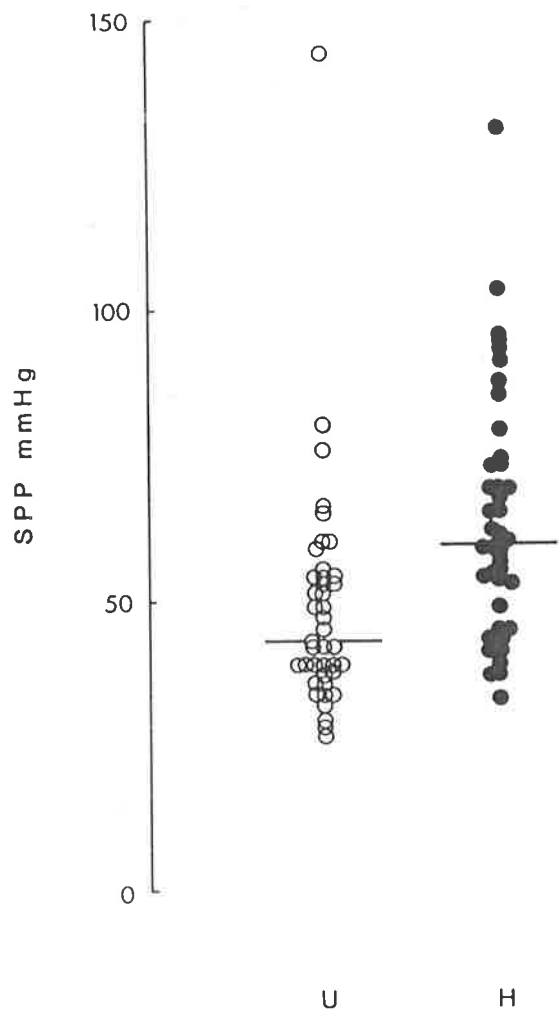


FIGURE 6.4

SPP in the unhealed (U) and healed (H) groups.

different ranges of SPP.

(5) Skin vascular resistance: The relationship between skin vascular resistance (SVR) and healing is shown in figure 6.5. The median SVR in the healed group was 688 (265-1534) units and in the unhealed group was 961 (255-2652) units ($p < 0.001$). Table 6.1 shows the percentage of patients healed for different ranges of SVR.

SPP, SVR, and healing

The relationship between SPP, SVR and healing is shown in figure 6.6. This figure is summarised in table 6.2 which shows the number of patients which were healed and unhealed depending on SPP and SVR.

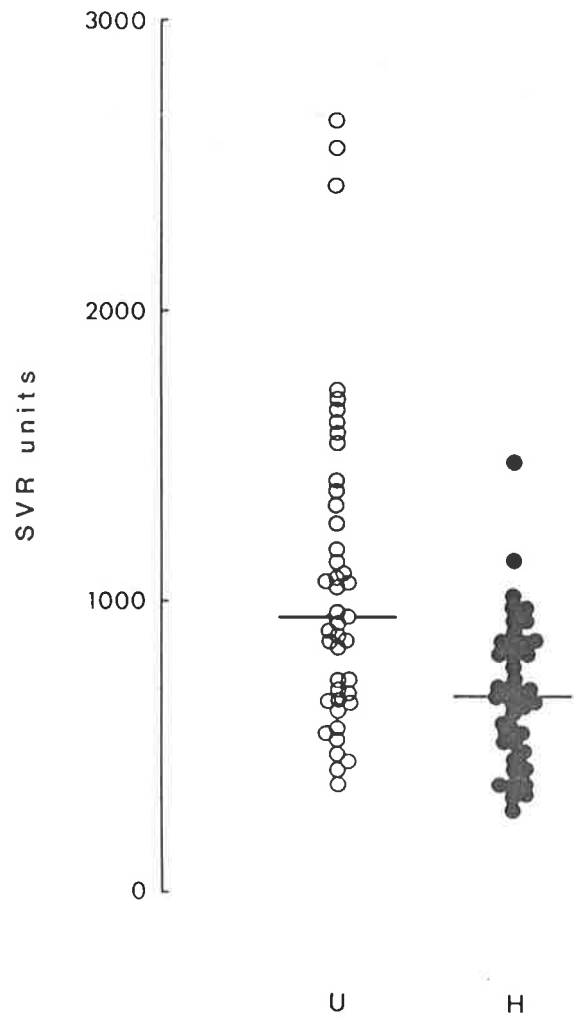


FIGURE 6.5

SVR in the unhealed (U) and healed (H) groups.

TABLE 6.1

Percentage of patients healed for different range of
kmax, SPP and SVR.

kmax (min ⁻¹)		
< .06	.06 - .08	>.08
18%	44%	86%

SPP (mmHg)		
< 40	40 - 50	> 50
18%	47%	67%

SVR (units)	
< 1000	> 1000
64%	13%

TABLE 6.2

Number of patients healed (H) and unhealed (U), in relation to SPP (mmHg) and SVR (units).

	<u>SPP</u>								
	< 40			40-50			> 50		
	H	U	%	H	U	%	H	U	%
< 1000	4	13	24	8	4	67	29	65	83
<u>SVR</u>									
> 1000	0	5	0	0	5	0	3	10	23

DISCUSSION

This study demonstrates that the estimation of SPP and SVR is useful in the assessment of lower limb ischaemia and as a predictor of healing of ischaemic lesions. The maximum washout rate of isotope (k_{max}), which indicates skin blood flow, is also a reliable predictor of healing. However, we have concentrated on the SPP and SVR as they provide useful information concerning the aetiology of the ischaemia, and a combination of these parameters gave the highest predictive value of healing.

The measurement of SPP and SVR provides information on the contribution of large and micro-vessel disease to the development and healing of an ischaemic lesion. Very little attention has been focused on the role of the microvessels in the pathogenesis of lower limb ischaemia. The presence of diabetic microangiopathy in the skin is well known (Goldeberg et al, 1959) but its significance is unknown. Nielsen (1973), Strandness, Priest and Gibbons (1964), Lo Gerfo and Coffman (1984) suggest that diabetic microangiopathy plays no part in the pathogenesis of skin

ischaemia. This study indicates that disease of the microvessel, as indicated by elevated SVR, is in fact very important in the development of skin ischaemia. The realisation of the importance of microvascular disease, and the availability of a simple diagnostic test to detect this disease, may aid in the understanding and treatment of lower limb ischaemia.

Apart from providing information about the severity and relative importance of peripheral vascular disease and microvascular disease the test has value as a predictor of healing of ischaemic lesions of the lower limb (figure 6.6, table 6.2). If the SPP < 40 mmHg then there is only a 18% chance of healing irrespective of the SVR. A SPP of 40 mmHg appears to be a critical level below which healing is unlikely. Previous studies have shown similar results (Holstein, 1973; Holstein, Sager and Lassen, 1979). In these patients a trial of conservative therapy is not indicated. We would recommend an arteriogram be performed to determine the suitability for reconstructive surgery. If this is not possible the patient may require a major amputation. This aggressive approach in this subgroup of

patients would greatly reduce long admissions to hospitals and reduce the incidence and morbidity associated with multiple distal amputations.

If the SPP lies between 40 and 50 mmHg there is a 67% chance of healing if the SVR is less than 1000 units. In these cases a trial of conservative therapy or local amputation is indicated before proceeding to arteriography. If the resistance is greater than 1000 units then healing is unlikely and arteriography is indicated.

If the SPP > 50 mmHg all patients deserve a trial of conservative therapy or local amputation, especially if the SVR < 1000 units, where there is a 83% chance of healing. If the resistance is greater than 1000 units the probability of healing is reduced to 23%. In these patients an early arteriogram may be performed if conservative therapy was unsuccessful.

This study has also demonstrated the influence of age, diabetes mellitus and hypertension on healing. With increasing age there is decrease in the healing potential of

an ischaemic lesion. Although this has been suspected by clinicians it has not been previously documented. There may be many factors contributing to age-related healing potential. These include changes in collagen metabolism and wound healing, structural change to the skin and changes to the blood vessels. In chapter 4 it was demonstrated that the SVR increased with age implying narrowing of the resistance vessels in the microcirculation. This increase in SVR resulted in a decrease in skin blood flow, therefore, providing a possible explanation for delayed healing seen in old age. It is beyond the scope of this study to analyse all age-related factors which may influence healing but it should be emphasised that age is a significant determinant of healing and should be considered when making clinical decisions.

The influence of diabetes mellitus on healing of ischaemic lesions of the foot is well known (Faris, 1975; Steer et al, 1983) and has been confirmed by this study. Hypertension also appears to have a significant influence on healing. Apart from patients with Martorell's hypertensive leg ulcers, which are a result of hypertensive microvascular

disease, there has been little in the literature which suggests that hypertension may influence the pathogenesis or healing of skin ischaemia. In this study we have found that hypertension significantly reduced the probability of healing. This is most probably related to hypertensive disease of the microcirculation.

This study has demonstrated that the measurement of SPP and SVR provides valuable information concerning the aetiology of lower limb ischaemia, the likelihood of healing of an ischaemic lesion and, therefore, allows a more rational approach to the management of these patients. It has also shown the importance of microvascular disease in the development of skin ischaemia. The influence of diabetes mellitus, hypertension and age on healing of an ischaemic lesion was also discussed.

CHAPTER 7

MATHEMATICAL ANALYSIS OF VARIABLES INFLUENCING HEALING OF ISCHAEMIC LESIONS OF THE LOWER LIMB

INTRODUCTION

The influence of age, diabetes, hypertension, SPP and SVR on healing of ischaemic lesions of the lower limb was discussed in the previous chapter. These variables are interrelated, for example, diabetes may influence the SVR and through this mechanism interfere with healing, and thus in the previous chapter it was not possible to determine the importance of each variable independent of the others. To investigate this problem the data in chapter 6 was analysed statistically and this analysis will form the basis of this chapter. The analysis was performed by Mr. Phil Leppard of the University of Adelaide Department of Statistics.

METHODS

A logistic regression analysis (Statistical Software Package BMDP 81, ULCA, USA) was used to analyse the variables age, presence of diabetes and/or hypertension, and the SPP and SVR values as predictors of healing of ischaemic lesions of the lower limb.

The logistic model in this situation was expressed mathematically as:

$$\text{Pr (lesion healed)} = 1/(1 + e^{-\alpha x})$$

where x was the vector of the patients variables mentioned above and α was a vector of unknown co-efficients which were estimated from the data.

The BMDP Statistical Software Package was used to estimate α and to then determine which components of α could be statistically considered zero. Likelihood ratio tests at the 5% level were used in this process. The inference following from such conclusion was that the corresponding variable was not statistically significant in predicting the healed/unhealed outcome. In other words, this software

package enabled the relative importance of each variable to be determined independently of the other variables.

RESULTS

The analysis indicated that only three variables were significant in predicting the outcome. These variables and their estimated co-efficients were age (co-efficient -0.90), SVR (co-efficient -0.005) and SPP (0.097). It should be noted that neither diabetes nor hypertension were significant variables. The negative co-efficient indicated that a high value for this variable reduced the likelihood of healing whereas the positive vector indicated an increased likelihood of healing. Although interaction terms were considered none were found to be significant.

Three separate goodness-of-fit tests were performed, the classic likelihood ratio, and tests developed by Hosmer and Brown all of which were included in the BMDP 81 package. None were significant at the 20% level.

With the numerical estimates obtained from the analysis it was possible to generate tables of the estimated probabilities of healing for different combinations of variables. These tables are contained in appendix 1.

Graphs (fig. 7.1-7.3) were drawn from these tables and are shown in the subsequent pages.

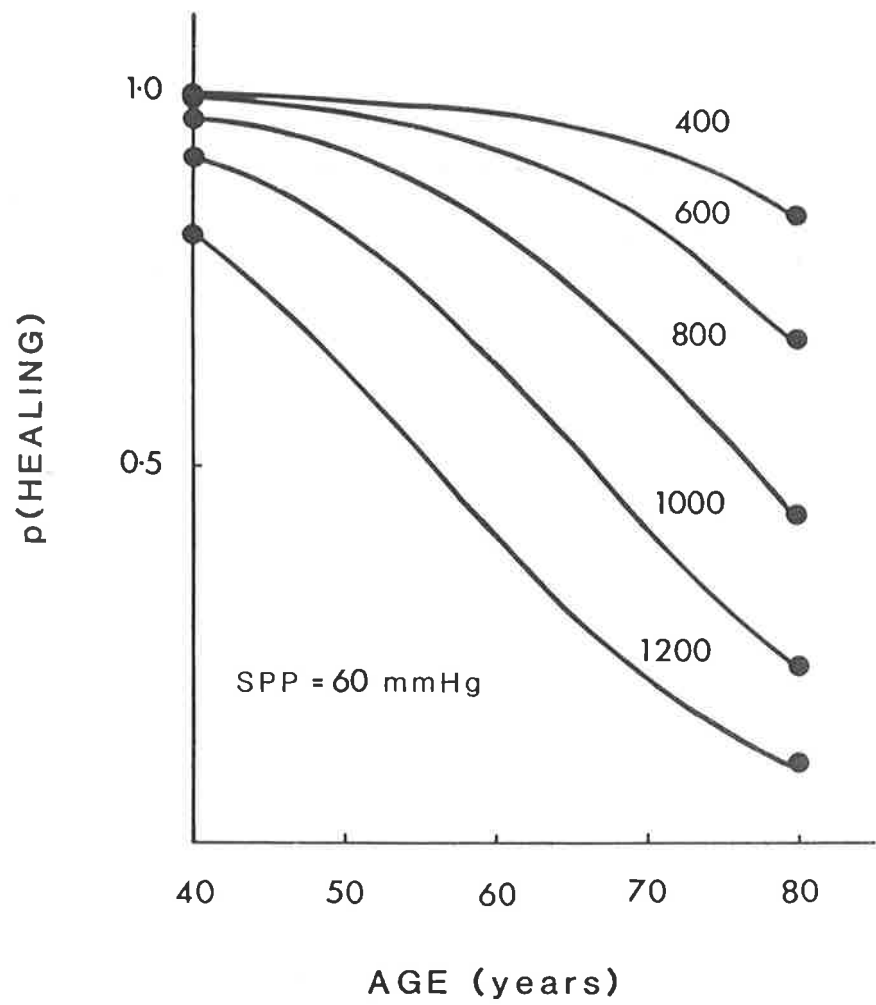


FIGURE 7.1

The effect of SVR (range 400 - 1200 units) and age on probability of healing at a fixed SPP (60 mmHg).

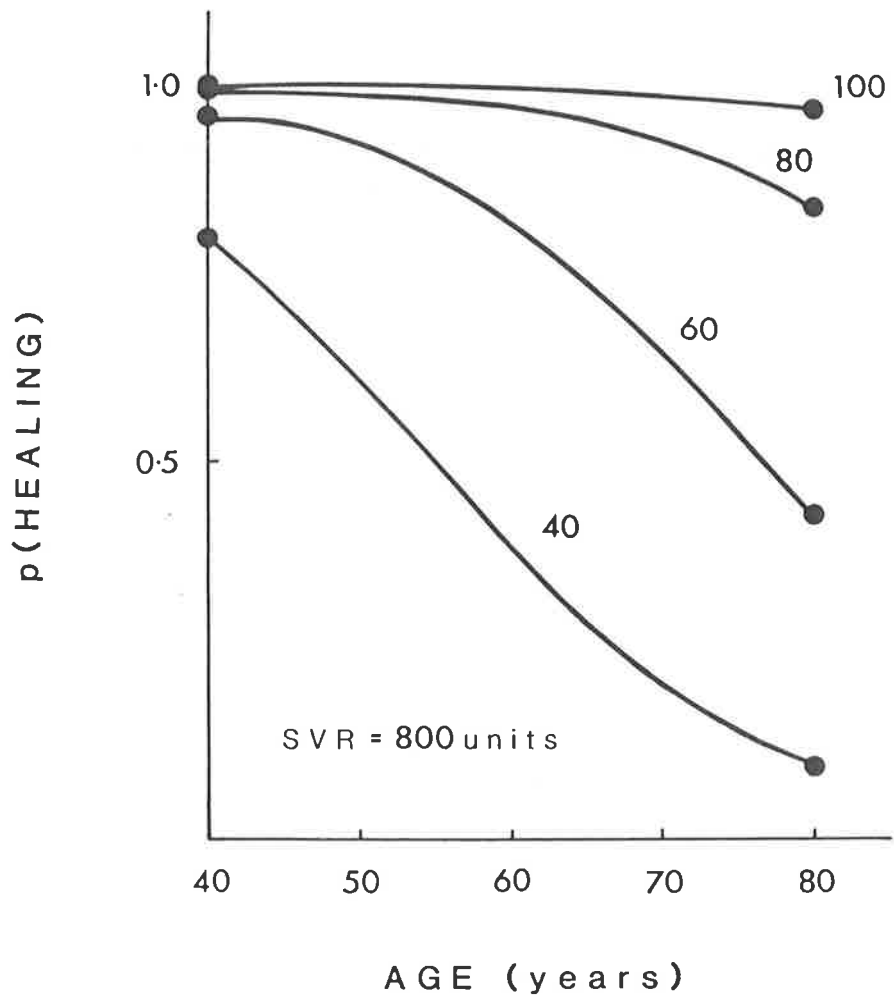


FIGURE 7.2

The effect of SPP (range 40 - 100 mmHg) and age on probability of healing at a fixed SVR of 800 units.

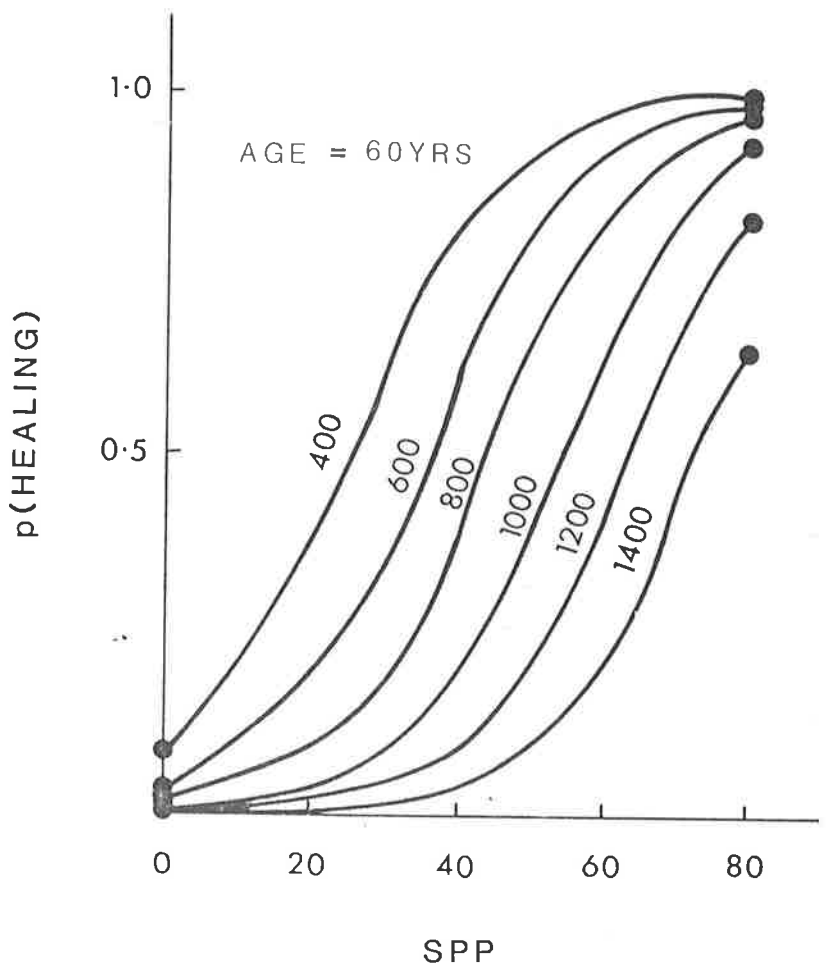


FIGURE 7.3

The effect of SPP and SVR (range 400 - 1400 units) on probability of healing. The age is constant at 60 years.

DISCUSSION

The most interesting result of this analysis was that neither diabetes nor hypertension per se were found to influence healing of ischaemic lesions of the lower limb. This indicates that the adverse affect of these two conditions on healing, which was discussed in chapter 6, was due to a complication not the disease itself. The most likely explanation is that the microvascular disease which is associated with these conditions was responsible for the impaired healing. Therefore, if the patient has either diabetes or hypertension and the SVR is normal, indicating that there is no microvascular disease, then there may be no added risk to healing.

The other variables analysed (age, SPP and SVR) were all important determinants of healing (fig. 7.1-7.3). The tables provide an easy method of assessing the likelihood of healing occurring and may thus be of benefit in patient management as along the lines discussed in chapter 6. That is, if the probability of healing is low as determined from the age, SPP and SVR then conservative management is not

indicated and early arteriography and surgery is indicated. Conversely, if the probability is high that healing will occur and conservative management is likely to succeed and unnecessary arteriography and surgery can be avoided. This rational approach to the management of patients with ischaemic lesions of the lower limb could reduce lengthy hospital admission along with patient mortality and morbidity.

CHAPTER 8

GENERAL DISCUSSION

INTRODUCTION

There have been two major obstacles to the rational assessment and management of patients with ulceration and gangrene in the lower limb. These have been:

- (i) the absence of suitable clinical test to determine the blood supply to the skin, and
- (ii) the inability to diagnose microvessel disease without taking biopsies.

This has had several consequences. Firstly, clinical decision making has been difficult and often inefficient resulting in inappropriate management. If the blood supply to the foot is sufficient, despite a major arterial occlusion, arterial reconstructive surgery is not necessary. On the other hand, patients with an insufficient blood supply will not heal without reconstructive surgery. Second, the assessment of the role of microvessel disease in

the aetiology of skin ischaemia has been based more on intuition than measurement.

The studies undertaken in this thesis have provided a rational scheme of management based on simple measurements and provide evidence to support the hypothesis that elevated skin vascular resistance is related to the degree of microvessel disease.

The Isotope Washout Technique

In this thesis a method has been developed which gives a quantitative estimate of skin perfusion pressure (SPP) and skin vascular resistance (SVR). This technique is based upon the isotope washout method introduced by Kety (1948; 1949). Lassen (1984) states that this is the most suitable method of measuring the blood flow in a specific vascular bed in vivo.

The method is based on the principle that when a freely diffusible isotope is injected into a tissue the rate of clearance of the isotope from the tissue is directly

proportional to the blood flow through that tissue. There have been a number of problems associated with this method since its introduction but these have largely been overcome in the last decade (Lassen, 1984).

By using an adaptation of this method it became possible to measure the blood pressure in skin and muscle (Lassen and Holstein, 1974) and these measurements were found to be useful in assessing amputation stump healing (Holstein, 1973; Holstein, Sager and Lassen, 1979). Unfortunately the general perception of isotopic methods is that they are complex and require sophisticated and expensive equipments. This is probably one of the reasons they have not received wide acceptance.

An important aspect of the method involves the production of vasodilatation of the vascular bed under study, because if the vessels are not maximally dilated it is possible that the vascular resistance may not indicate structural features of the microvessels but rather factors which alter vascular muscle tone, such as sympathetic nervous tone, humoral influences and local metabolic or

myogenic factors. By eliminating these factors by paralyzing the vascular smooth muscle it is likely that alterations in vessel lumen ratio, and thus differing resistances, are due ~~ue~~ to structural changes to these vessels. Therefore, by measuring the vascular resistance in vasodilated skin it is theoretically possible to detect and quantify disease of these microvessels as occurs in diabetes mellitus and chronic hypertension.

To determine to most suitable vasodilating agent a range of agents was studied in an animal model and it was found that two agents, histamine and nitroprusside, produced a similar degree of vasodilatation. However, it was found that in the clinical setting the dose of nitroprusside had to be increased to have similar potency to histamine. This may be explained by differing sensitivities between human and rabbit vessels to the various agents. This question of sensitivities is an important one as it is possible that in different disease states, such as diabetes mellitus and hypertension, there may also be different sensitivity to vasodilators which may interfere with the interpretation of vascular resistances. This is one area which will need to

be studied in the future.

The meaning of SPP

The skin blood pressure or skin perfusion pressure (SPP) has been found to be similar to the mean arterial pressure (Holstein et al, 1977; 1983). In patients with peripheral vascular disease (PVD) the SPP was reduced in proportion to the severity of the PVD. The SPP appears to be an 'indirect wedge pressure' measurement of the pressure of the main artery supplying the area under study and, therefore, is very useful as an objective, quantitative indicator of PVD. SPP is superior to ankle pressure for assessing PVD as ankle pressure may be falsely high when rigidity of the medium sized arteries reduces their compressability. This is a particular problem in diabetic patients, many of whom have calcification of the tunica media. In some patients the arteries cannot be compressed and ankle pressure recordings may be as high as 250-300 mmHg despite an obvious ischaemic foot. These patients are easy to recognise. The problem arises when the arteries are not incompressible but when they are compressible but more rigid than normal thus resulting in ankle pressures which appear compatible with the clinical setting but in fact may be

significantly higher than the actual blood pressure thus confusing the clinical picture. The comparison of ankle pressures with SPP in patients with PVD demonstrated that the ankle pressures were higher than the SPP for the whole range of pressures in diabetic compared to nondiabetic patients confirming the problem of an increased rigidity of medium sized arteries, not just of incompressible arteries, in diabetic patients. Therefore, the SPP is probably a better indicator of the severity of PVD in diabetic patients than ankle pressures.

The Meaning of SVR

The skin vascular resistance (SVR) has been measured indirectly to study the significance of the microcirculation in the pathogenesis of hypertension (Henriksen et al, 1981) and in the study of autoregulation of blood flow (Henriksen et al, 1973). In a pilot study Faris and Lassen (1982) introduced a direct measure of SVR and this method formed the basis of the technique described in this thesis.

In individuals with normal limb circulation, i.e. in

subjects free from peripheral vascular disease, diabetes mellitus and hypertension, the SVR appears to be similar when measured by either indirect or direct method. However, in patients with PVD it is not possible to estimate the SVR in the periphery as the mean perfusion pressure can not easily be calculated and, therefore, the indirect method is the only method available.

The concept of an elevated SVR indicating microvascular disease in diabetes and hypertension has been indirectly confirmed in this thesis by the finding of an increased SVR in these conditions when both nitroprusside and histamine were used as the vasodilating agent. In the initial study using histamine there was a large degree of heterogeneity in the groups studied in that the subjects may have had PVD, mild noninsulin-dependant diabetes or severe insulin-dependant diabetes. Despite this there was an increase in the SVR in the diabetic and hypertensive groups. The second study, using nitroprusside as the vasodilator, more homogeneous groups were studied. It was demonstrated that in long-standing hypertensive patients and in diabetic patients with severe retinopathy requiring laser therapy the

SVR was much higher than age matched subjects free of hypertension and diabetic patients without significant retinopathy.

Therefore, in subjects with long standing hypertension or in diabetics with severe retinopathy, in whom microvascular disease is more likely, there was an elevated SVR. It is not possible, however, to state conclusively that this elevated SVR is due to the microangiopathy. For example, in the diseased states there might be different sensitivities to the vasodilating agents thus altering the degree of relaxation in the vessel walls. This is unlikely as there were similar results when two different agents were used. Another possibility is that in the disease states there might be an alteration in the density of the microvessels in the skin. This has been demonstrated in hypertensive rats (Hutchins and Dornell, 1974) but not in man. These areas require further study.

The only way to determine the relationship between the SVR and microvascular disease is to perform histological studies. The limited histological studies performed in this

thesis did suggest than an elevated SVR did indicate microvascular disease but the small numbers examined do not allow a definite conclusion. This is another area which requires further study, perhaps in an animal model.

The Interpretation of SPP and SVR

The interpretation of SPP and SVR is dependent upon the age of the patient and the site studied. The SPP increased with age in parallel to the increase in mean arterial pressure. The SVR also increased with age reflecting age related changes to the microvessels (Braverman and Fonferko, 1982) thus offering a possible explanation for the reduced healing potential of ischaemic lesions in the elderly.

The SPP and SVR were also higher in the calf compared to the foot. Again the explanation for the higher SPP is relatively easy. In the foot the arterial pressure is reduced because of the passage of blood through the medium sized and small arteries of the foot thus reducing the SPP. However, the explanation of the higher SVR in the calf is not as simple. A possible explanation is that the

microvessels compensate for the higher arterial pressure by hypertrophy of the media and intima, as occurs in chronic essential hypertension, with a resultant reduction of the vessel lumen. Another possible explanation is that there is a reduced vessel density in the skin of the calf compared to the foot and with the reduced density there is a corresponding increase in the vascular resistance. It was interesting to note that the skin blood flow was the same in the calf and the foot.

Clinical Significance of SPP and SVR

By estimating both SPP and SVR in patients with ischaemic lesions of the lower limb it is possible to determine the severity and the aetiology of the ischaemia and, therefore, be able to approach the management of these patients in a rational way. The SPP indicates the severity of atherosclerotic arterial disease, i.e. disease of the large and medium sized arteries, while the SVR indicates the presence and severity of microvascular disease.

One of the interesting and most important findings in this thesis is the importance of microvascular disease in the aetiology of skin ischaemia. A common belief is that diabetic microvascular disease is not a significant factor in skin ischaemia (Niesen, 1972; Lo Gerfo and Coffman, 1984). The microvascular disease associated with hypertension has largely been ignored when considering ischaemic disease of the legs except in the case of Martorell's hypertensive leg ulcers where it was concluded that the ulcers are secondary to hypertensive microvascular disease (Martorell, 1945; Hines and Farber, 1946). The

studies in this thesis demonstrate that microvascular disease plays a significant role in lower limb ischaemia in both diabetes and hypertension.

The measurement of the SPP and SVR enabled the classification of patients with ischaemic ulceration and gangrene into three groups:

(i) Reduced SPP and normal SVR.

These are the conventional atherosclerotic patients without evidence of microvascular disease. This group have the best prognosis as they are potentially treatable by arterial surgery.

(ii) Reduced SPP and elevated SVR.

This group has both atherosclerotic disease of the main arteries and microvascular disease. They would also benefit from arterial surgery but the outcome may be hindered by the microvascular disease.

(iii) Normal SPP and elevated SVR.

In this group the ischaemia is due solely to microvascular disease. This group includes

patients with Martorell's ulcers and some diabetic patients. Arterial surgery has little to offer in this group. However, a possible mechanism of improving microvascular function is the manipulation of the haemorrheology by drugs which improve blood viscosity, red blood cell fragility or oxygen extraction by the tissues.

It is interesting to note that when the data is analysed mathematically the detrimental effect hypertension and diabetes mellitus has on healing appears to be related mainly to their associated microvascular disease. This implies that in diabetic patients, if blood sugar levels and infection are controlled, and there is no microvascular disease then the likelihood of healing is similar to nondiabetic patients.

Once the SPP and SVR are known an objective plan of management is possible. If the SPP is less than 40 mmHg then healing by conservative measures is most unlikely. These patients warrant early arteriography and reconstructive surgery if possible. If reconstructive

surgery is not possible then serious thought should be given to an early major amputation. If the SPP is greater than 50 mmHg a trial of conservative management or local amputation is indicated irrespective of the SVR. However, if the SVR is greater than 1000 units healing is less likely to occur and, therefore, a more aggressive approach is justified. If the SPP is between 40 and 50 mmHg and SVR greater than 1000 units early arteriography and surgery is indicated, whereas, if the SVR is less than 1000 units a trial of conservative management is indicated. By rationalising the management of patients along these lines lengthy hospital stays and unnecessary operations can be avoided reducing costs and patient mortality and morbidity.

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APPENDIX:

The calculated probability of healing
at varying SVR and SPP at ages ranging
from 45 - 80 years.

See Chapter 7 for details.

ADDENDUM

Page 5 Add to the first paragraph

The changes in blood viscosity under conditions of irregular flow have only a small effect in the microcirculation compared with changes in flow caused by alterations in tone in the resistance vessels.

Page 11 Add to the first paragraph

However, capillary endothelial swelling and contraction of endothelial cells associated with histamine administration may be significant factors

Page 75 Add to end of page

The difference in age between groups II and III was not significant

Page 76 Add after the second sentence

This may have produced falsely low readings of blood pressure in patients with atheromatous narrowing of the proximal subclavian artery.