CELL MEMBRANE PHOSPHOLIPIDS IN THE GENESIS
AND MAINTENANCE OF HYPERTENSION

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CHAPTER 1

INTRODUCTION
INTRODUCTION

Between ten and twenty percent of the United Kingdom (UK) adult population may be classified as having raised blood pressure (hypertension) using conventional clinical criteria (diastolic blood pressure greater than 95mmHg) and in consequence these people have an increased risk of developing cardiac and cerebrovascular diseases (Pickering 1968). The detection of a primary condition initiating blood pressure elevation such as renal, endocrine or neurological disease is possible in less than two percent of such patients, and the remainder are classified as having 'essential hypertension'.

Essential hypertension tends to occur in families (Platt 1947) and arterial pressure has been shown to be similar in first degree relatives across the spectrum of pressure values (Miall and Oldham 1958, 1963). Although some of the resemblance in blood relatives may be ascribed to shared environmental influences studies in monozygotic and dizygotic twins support the concept that genetic factors are an important determinant of arterial pressure (Pickering 1968). Thus even if a search for a solitary lesion in this disease is unlikely to be successful, investigations directed towards discovering the mechanisms that generate the rise in blood pressure are still of immense value and enormous therapeutic potential.

STRUCTURAL ABNORMALITIES AND RESISTANCE

The fundamental abnormality demonstrated in patients with longstanding essential hypertension is an increased resistance to blood flow in the peripheral vasculature at a time when the cardiac output is normal (Lund-Johansen 1980). The cause of this
may be due to an increased activity of neurohumoral mechanisms initiating vasoconstriction (Abboud 1980); however, when the influence of these mechanisms is abolished some increased resistance remains and therefore a part of this is attributable to a structural abnormality (Folkow 1978, 1982). More than a century ago structural changes affecting the ventricular wall of the left side of the heart, conduit arteries and small precapillary resistance arteries were documented in hypertension (Bright 1836; Johnson 1868); these abnormalities were absent in veins. Although these are the obvious, consistent characteristics of chronic hypertension, their haemodynamic consequences have been appreciated only comparatively recently. According to the law of Poiseuille, resistance to blood flow is proportional to the fourth power of the radius of the vessel lumen; therefore, it follows that small changes in the wall-to-lumen ratio will have a large effect upon resistance. Initiation of vascular contraction is associated with shortening of smooth muscle cells and narrowing of the lumen. In the presence of a structural excess, an exaggerated reduction in the luminal diameter will result from a given contraction (Folkow et al. 1973; Folkow 1982). Thus dose-response curves to pressor hormones such as noradrenaline or angiotensin II are steeper in the presence of vascular hypertrophy because the resistance to blood flow is potentiated (Folkow et al. 1973). There is little evidence to suggest vascular hypertrophy precedes increased arterial pressure in patients with secondary or essential hypertension, or in rats if the hypertension is experimentally induced or genetically determined. Therefore, it is unlikely that it is the primary mechanism, but may be secondary to the increased load placed upon
the vasculature by the initiating process, ultimately maintaining hypertension chronically.

**ABNORMALITIES OF CELL STRUCTURE**

There is evidence for a fundamental defect in the cell plasma membrane in essential hypertension (Swales 1982). Much of the work has been carried out in the erythrocyte: although this tissue has no obvious role in the development of hypertension, it has been studied in the assumption that it reflects abnormalities present in other tissues and because it is easily accessible. Red cell membrane viscosity and calcium binding are abnormal and in platelets free intracellular calcium has been shown to be increased in essential hypertension (Postnov et al. 1980; Orlov and Postnov 1982; Erne et al. 1984), and genetically hypertension-prone rats (Postnov et al. 1981; Aragon et al. 1982; Orlov et al. 1982). These two abnormalities may be closely associated because calcium binding to the erythrocyte membrane is reduced in hypertension (Gulak et al. 1972; Postnov et al. 1977; Postnov et al. 1979; Devynck et al. 1981) and the sialic acid component of glycoproteins and glycolipids, an important determinant of membrane calcium binding and fluidity, is reduced (Reznikova et al. 1984). In this context, membrane phospholipids may be implicated; such lipids account for approximately 20% of calcium binding by erythrocyte membranes (Forstner and Mannery 1971; Duffy and Schwarz 1973), and a physiological role in this regard has been ascribed to both phosphatidylserine (Long and Mouat 1971) and the polyphosphoinositides (Buckley and Hawthorne 1972; Michell 1975). The heavily phosphorylated derivatives of phosphatidylinositol are present predominantly on the inner

ELECTROLYTE TRANSPORT CELL STRUCTURE AND HYPERTENSION

Opposing the passive movement of sodium and potassium down their respective concentration gradients across cell plasma membranes, sodium/potassium-ATPase, a membrane bound protein complex, moves potassium inwards and sodium outwards and is responsible for maintaining the potential difference across cell membranes. This transport system, known as the sodium pump, is specifically inhibited by the glycoside ouabain and has been studied closely in hypertension. Although conflicting data have been obtained in the erythrocyte (Swales 1982), reduced sodium/potassium-ATPase activity and an increase in intracellular sodium have consistently been reported in leucocytes in hypertensives (Edmundson et al. 1975; Araoye et al. 1978; Heagerty et al. 1982). These cells are likely to resemble more closely the physiology of nucleated cells in other tissues. Other sodium and potassium transport processes located in the plasma membrane have been investigated in essential hypertension. Sodium-potassium counter-transport which exchanges external sodium for internal potassium ions, and sodium-potassium co-transport which moves sodium and potassium ions together across the cell membrane have been shown to be abnormal in erythrocytes by some workers (Garay et al. 1980; Canessa et al. 1980; 1981), but not by others (Swarts et al. 1981; Davidson et al. 1982; Duhm et al. 1982).
Similar studies of membrane electrolyte transport have been carried out in the rat. The majority of investigators have based their experiments in genetic hypertension on an inbred strain, on the grounds that this may provide a better model of essential hypertension in man than models which require the administration of mineralocorticoids or renal manipulation. Although caution is required in extrapolating data from animal models to man, the demonstration of similar changes in different tissues of genetically hypertensive rats and patients with essential hypertension may lead to an understanding of the mechanisms responsible for blood pressure elevation in both species.

In genetic and experimental hypertension in the rat, a similar and consistent pattern of change has been demonstrated in erythrocytes and in blood vessels. Membrane sodium and potassium permeability is increased (Jones 1974; Jones and Hart 1975; Friedman and Friedman 1976; Friedman et al. 1976; Postnov et al. 1976; Yamori et al. 1977; Garwitz and Jones 1982), this is associated with increased activity of the sodium pump as evidenced by increased absolute efflux rates for sodium (Webb and Bohr 1979; Jones 1981). Indeed, only when vascular smooth muscle has been incubated in plasma has inhibition of the sodium pump been demonstrated in volume expanded models of experimental hypertension in the rat (Pamnani et al. 1981). The presence of similar abnormalities in human hypertensives and their normotensive offspring (Meyer et al. 1981; Heagerty et al. 1982; Woods et al. 1982), and in genetic and non volume-expanded experimental hypertension in the rat suggest changes in cation transport observed in essential hypertension to be genetically determined, related to hypertension but not a reflection of
Membrane ion transport is a fundamental participant in smooth muscle excitation-contraction coupling, and hence vascular resistance. It is likely that abnormalities in sodium and potassium transport are in some way related to the process which elevates blood pressure. Depolarisation of the smooth muscle cell membrane induced by an increased turnover of these ions may induce an enhanced responsiveness to vasoconstrictor stimuli (Jones 1974; Hermusmeyer 1976). Contraction in vascular smooth muscle is mediated by an increase in cytoplasmic ionised calcium in the region of the contractile protein (Bolton 1979), a change produced by entry of calcium ions into the cell, the release of calcium from intracellular stores and the liberation of calcium bound by the inner leaflet of the plasma membrane. The link between abnormal membrane monovalent cation transport in essential hypertension and altered excitation-contraction coupling in vascular smooth muscle was addressed by Blaustein a decade ago. Blaustein (1977) pointed to evidence for a sodium-calcium transport mechanism in cardiac muscle exchanging intracellular calcium for external sodium ions. Inhibition of this exchange process by a reduction in sodium/potassium-ATPase activity and concomitant elevation in intracellular sodium content would lead to an increase in intracellular calcium, and if present in vascular smooth muscle may produce enhanced smooth muscle tension and an increase in peripheral vascular resistance. This hypothesis has been developed further and appears more attractive in the light of a proposed circulating inhibitor of the sodium pump such as that postulated by Haddy et al. (1978), present in the blood of individuals with and without essential
hypertension when dietary sodium is increased.

de-Wardener and MacGregor (1980) have emphasised the importance of the experiments of Dahl's group (Dahl et al. 1969) and proposed that hypertensive individuals have a genetically abnormal kidney unable to excrete excess dietary sodium, leading to expansion of blood volume and the release of the postulated natriuretic hormone to promote renal sodium loss. The ouabain like properties of the natriuretic hormone depress sodium/potassium-ATPase activity leading to an increase in peripheral resistance through steps outlined in Blaustein's original hypothesis. de-Wardener and MacGregor's 'inhibitor hypothesis' appears to have a number of problems; it is doubtful whether such a circulating factor, as yet uncharacterised, has any role in raising blood pressure acutely as inhibition of the sodium pump can be demonstrated in a variety of disease states without associated hypertension (Swales 1975). Also, there is no evidence to suggest volume expansion in patients with essential hypertension (Bing and Smith 1981); nor is there any strong evidence of a sodium-calcium exchange system to be active in vascular smooth muscle except during extreme alterations to the extracellular sodium concentration (Van Breemen et al. 1979; Brading and Lategan 1985).

Calcium extrusion is reduced in erythrocytes in hypertension in the presence of calmodulin (Orlov et al. 1983), and studies in biological and reconstituted membrane systems show that calcium/magnesium-ATPase, sodium/potassium-ATPase and other membrane proteins are sensitive to manipulations effecting changes in membrane fluidity (Kimelberg and Pathadjopoulos 1972; Cooper 1977; Swales 1983; Yeagle 1983). Thus disturbances in cell
membrane structure and in particular glycoprotein/glycolipid and phospholipid membrane components might lead to a change in a variety of cellular functions and explain the changes in membrane fluidity and calcium affinity observed in hypertension. Moreover, they could cause an increase in cytosolic free calcium and as a result change the membrane permeability to sodium, although the intracellular concentration needed for this to occur is high (Romero and Whittam 1977). The sodium pump would then be stimulated by the increase in intracellular sodium ions (Postnov et al. 1977), and according to such an hypothesis changes in cellular handling of sodium, potassium and calcium would lead to increased vascular resistance as a result of a fundamental genetic disturbance in membrane structure.

In the light of the lack of good evidence to support the 'salt hypothesis', a structural disturbance of the cell plasma membrane in hypertension, otherwise known as the 'membrane hypothesis' as originally proposed by Swales (1982), becomes attractive and has gained interest following the recent description of cellular signalling systems that mediate between changes in intracellular ion transport, contraction and growth on the one hand and receptor mediated activation of smooth muscle on the other. Such a signalling system is the phosphoinositide second messenger system and is activated by hydrolysis of inositol-containing phospholipids (described in detail below) present in the cell plasma membrane whose esterified fatty acids may play a crucial role in determining the nature of the agonist-induced response and in the resistance vasculature could occupy a central position in the maintenance of arterial pressure. Thus a link between dietary fat intake which influences membrane fatty
acid composition and hence phospholipid function becomes very attractive in a disease characterised by smooth muscle proliferation and luminal narrowing.

**DIETARY MODULATION**

The importance of the possible disturbances in membrane function in hypertension resides in the fact that modifications in the structure of the cell membrane may be implicated in the hypotensive effect of a lacto-ovo-vegetarian diet (Iacono et al. 1982); this diet may also beneficially affect serum cholesterol (Iacono et al. 1975) and triglyceride concentrations (Judd et al. 1981), and has been associated with a reduced vascular reactivity (Rouse et al. 1984) and a possible fall in the incidence of cardiovascular disease (Keys 1956; Logan et al. 1978; Wood et al. 1984), and therefore may provide an insight into the possible link between membrane composition and blood pressure control mechanisms.

**VEGETARIANS**

A number of epidemiological and clinical dietary intervention studies have investigated how such a diet might modify blood pressure: reports of a reduced blood pressure found among vegetarian monks of all ages (Saile 1930; Raab and Friedman 1936; Groen et al. 1962; Sacks et al. 1974), and the pressor effect of adding meat to the diets of established vegetarians (Donaldson 1926; Sacks et al. 1974; Sacks et al. 1981) provide the basis for the more recent controlled clinical studies in which vegetarian diets have been used to attenuate blood pressure in normotensive subjects (Rouse et al. 1983a; Rouse et al. 1983b;
A lacto-ovo-vegetarian diet is associated with an increased intake of dietary fibre, polyunsaturated fat, magnesium, potassium, vitamins C and E, a significantly reduced intake of total fat, saturated fat, cholesterol, protein and vitamin B₁₂, and similar amounts of energy, total carbohydrate, sodium and calcium (Rouse et al. 1983b; Rouse et al. 1983c). Such a high degree of co-variability of nutrient intakes makes it difficult to isolate the effect of any one from that of others varying with a change to a vegetarian diet. Dietary fibre intake may affect the absorption of other nutrients across the gut (Kelsay 1978), and there is some evidence linking dietary potassium (Iimura et al. 1981; Khaw and Thom 1982; MacGregor et al. 1982), and magnesium (Altura et al. 1981) with hypertension. However, the differences in intake of these ions is small and alone unlikely to account for the observed changes in arterial pressure (Kelsay 1978; 1982). Similarly, there is no compelling evidence to suggest a role for dietary protein, vitamin B₁₂ or for the antioxidants, vitamin C or vitamin E. When carefully controlling for variables associated with a change to a vegetarian diet, one hypotensive mechanism which might be implicated is the change in dietary fat; an increased intake of lipids whose fatty acids contain more than one double bond (polyunsaturated fatty acids) has been proposed as the means by which a reduction in blood pressure is achieved (Kim 1975), although formal testing of this hypothesis has failed to prove this (Beilin 1987).

Endogenous fatty acid synthesis in mammals is limited to providing acyl chains containing no double bonds (saturated) or a single double bond (monounsaturated). Polyunsaturated fatty acids
(PUFA) are necessary for normal membrane function because they are a crucial determinant of membrane fluidity, and obtained exclusively from the diet. These fats have been termed 'essential fatty acids' and are found esterified in triglyceride, phospholipids and cholesterol esters. Vegetable oils and marine foods are major contributors of essential fatty acids to the diet and predominate in PUFA in which the first unsaturated bond is found at the sixth (n-6) and the third (n-3) molecular carbon atom from the methyl terminal respectively. Vegetable oil n-6 PUFA are almost exclusively molecules containing eighteen carbon atoms comprising linoleic with two, and gamma linolenic which has three unsaturated bonds. These fatty acids undergo de-novo chain elongation and further desaturation to produce two twenty carbon atom molecules, dihomo gamma linolenic acid containing three unsaturated bonds and arachidonic acid containing four unsaturated bonds. Vegetable oils also contain trace amounts of n-3 PUFA in the form of alpha linolenic acid which differs from gamma linolenic acid only in the position of the first unsaturated bond. Marine fish oil, n-3 PUFA, is dominated by two fatty acids, eicosapentanoic acid a twenty carbon atom molecule containing five double bonds, and docosapentanoic acid which has twenty two carbon atoms and six unsaturated bonds (see figure 1). Twenty carbon atom PUFA molecules of both n-6 and n-3 series present in mammalian tissue lipids are precursors in the synthesis of many prostaglandins and leukotrienes catalyzed by cyclooxygenase and lipoxygenase enzymes respectively (Wardle 1985). No interconversion of PUFA between the n-6 and the n-3 series takes place; however a state of competition exists between twenty carbon atom PUFA of both series for cyclooxygenase, and as
arachidonic acid is a better substrate for this enzyme it is more rapidly converted to its cyclic endoperoxide (Needleman et al. 1979; Hamberg 1980) (see figure 2). Strong evidence of a link between dietary n-3 PUFA and a reduced incidence of atherosclerotic disease has prompted much research into platelet activation and n-3 PUFA nutrition (Sanders 1983). There is little evidence to suggest n-3 PUFA has any influence upon blood pressure homeostasis by way of cyclooxygenase derived products; however, these highly unsaturated long chain eicosanoids may influence membrane fluidity in tissues directly involved in the regulation of arterial pressure (Lorenz et al. 1983).

MANIPULATION OF DIETARY FAT

On average the UK diet contains 97.4g fat per day and this includes 8.2g n-6 PUFA, 1.3g n-3 PUFA and 48.8g saturated fatty acids (Peattie et al. 1983). Recent dietary intervention studies designed to evaluate the contribution of n-6 PUFA to the hypotensive role of a vegetarian diet have utilised modifications to the dietary weight ratio of n-6 PUFA to saturated fat (P/S ratio). This normally lies at 0.2 to 0.25 in a UK diet but in a vegetarian diet this ratio is around 0.8 to 1.0; such high ratios have been achieved in study diets by exchanging saturated fat for PUFA without altering the total caloric intake. Iacono et al. (1975) obtained a reduction in arterial pressure of 13mmHg systolic and 7mmHg diastolic following a 40 day feeding programme with a low fat diet with a P/S ratio of 1.0 in a study in normotensive American men with a pre-study dietary P/S ratio of 0.3. When the fat content was increased by 60% to the level of the typical United States diet and the P/S ratio maintained,
a similar fall in blood pressure occurred which returned to pre-
study levels within 33 days following the resumption of normal
diet. In a further study Iacono et al. (1975) has shown that the
hypotensive effect of increasing the dietary P/S ratio to 1.0
could be reversed by changing to an iso fat-calorific diet with a
P/S ratio of 0.3. Other workers have reported similar responses
in subjects fed diets with high P/S ratios. Rouse et al. (1983a)
reported a modest reduction in pressure when normotensive
subjects changed from an omnivore diet with a P/S ratio of 0.4 to
a vegetarian diet with a P/S ratio of 0.8. A 6mmHg systolic and
3mmHg diastolic fall in pressure was reported within 6 weeks in a
group of normotensive Australians. The magnitude of the increase
in P/S ratio appears to determine the extent of the reduction in
blood pressure but it should be made clear that this is not
related to the lipid content of the diet. Groups of normotensive
and hypertensive Finns showed a 9mmHg reduction systolic and
8mmHg reduction in diastolic pressure when changing from a
dietary P/S ratio of 0.2 to that of 1.0, and again blood
pressures returned to pre-study levels on return to a normal diet
(Puska et al. 1983). The pressor effect of a reduction in dietary
P/S ratio has recently been reported in a population of Italian
subjects (Strazzullo et al. 1986), a small but significant
increase of 4mmHg in systolic blood pressure appeared to parallel
the modest change in dietary P/S ratio from 0.4 to 0.2.

In adipose tissue, which reflects long-term dietary fat
intake, there have been reports of significant negative
correlations between linoleic acid content, blood pressure (Oster
et al. 1979; Wood et al. 1987) and cardiovascular disease (Wood
et al. 1984; 1987) in normotensive individuals, suggesting that
dietary $n^6$ PUFA may play a role in cardiovascular homeostasis. Iacono et al. (1975) have reported changes in indices of platelet aggregation when feeding high P/S ratio diets; this group and others suggest the balance of tissue prostaglandins is influenced by a change in dietary linoleate which may modify the rate of conversion of linoleic acid to the dienoic prostaglandins via tissue arachidonic acid, a change which may have a beneficial influence on blood pressure (Galli et al. 1980; Galli 1980; Paoletti et al. 1981).

Thromboxane, a potent platelet aggregating agent and vasoconstrictor (Hamberg et al. 1975), and prostacyclin, an inhibitor of aggregation and a vasodilator which reverses the action of thromboxane (Gryglewski et al. 1976), are both formed ultimately from dietary linoleate; a modification of the levels of these agents in the kidney and in blood vessels may modify the renal excretion of sodium and water and influence resistance to blood flow in the peripheral vasculature.

To address the task of evaluating to what extent the relatively minor component of dietary fat, linoleic acid has on blood pressure homeostasis and to evaluate the possible therapeutic value of diets rich in $n^6$ PUFA, studies in humans have utilised control diets supplemented with vegetable oils which have characteristic fatty acid profiles.

**DIETARY LINOLEIC ACID SUPPLEMENTATION IN HUMANS AND EXPERIMENTAL HYPERTENSION**

Vergrossen et al. (1978) was able to influence blood pressure with a moderate increase in dietary linoleate in men and women given sunflower oil margarine containing 54% linoleic acid,
sunflower oil for cooking and biscuits containing 43% weight to volume linoleic acid. No other attempts were made to alter normal dietary habits and a significant reduction in diastolic blood pressure was seen after 4 weeks, which returned to baseline levels when subjects resumed their normal diet.

Other groups have supplemented normal diets with vegetable oils using a more controlled protocol. Normotensive and mildly hypertensive individuals were given gelatin-coated capsules containing either a placebo substance of starch or paraffin, or capsules containing safflower oil, a vegetable oil which contains 72% linoleic acid by weight, in randomised double blind studies. A significant reduction in blood pressure was observed in both normotensive and hypertensive subjects (Fleishman et al. 1979; Rao et al. 1981). These results in human subjects suggest a possible role for dietary linoleic acid as a hypotensive agent in vegetarian diets. However, the mode of action is far from clear.

Investigations directed at manipulating one of several nutrients necessary to evaluate the relationship between diet and blood pressure can be difficult to perform in human subjects as it is necessary to follow very strict dietary regimes. It is possible to carry out more closely controlled investigations in experimental animals, and in the rat, a variety of experimental models of hypertension have been utilised. In normotensive Wistar and Sprague-Dawley rats MacDonald et al. (1981) and Smith-Barbaro et al. (1980) have shown a highly significant decrease in systolic blood pressure following nine weeks of feeding a diet containing 9.4% linoleic acid compared with rats fed a diet containing 0.41% linoleic acid. Similarly, rats on a diet rich in linoleate have been shown to have a lower blood pressure in
response to drinking 1.5% and 8% sodium chloride than rats fed a control or normal chow diet (Tribe et al. 1976; Ten Hoor and Van De Graaff 1978; Smith-Barbaro et al. 1981). These diets have also been shown to delay the onset of hypertension in Dahl salt-sensitive rats given 4% sodium chloride to drink (Tobian et al. 1982). However, a number of workers have been unable to prevent the development of raised blood pressure in neurogenic (Tribe et al. 1976a), renal (Croft et al. 1984; Codde et al. 1984) or genetically hypertensive rats (Singer et al. 1982). This latter group of workers began feeding the spontaneously hypertensive rat (SHR) a 15% fat diet containing 7.4% linoleate at 4 weeks of age. After 22 weeks of this diet blood pressures were not different from those in a group of SHR fed a 15% fat diet containing 1.23% linoleate, and were 12.3KPa higher than a group of normotensive Wistar control rats (Singer et al. 1982). However, hypertension was prevented in male SHR if a linoleic acid rich feeding programme was begun in pregnant dams during the last week of gestation and continued in the offspring (Hoffman 1982). In volume-expanded and neurogenic models, in addition to the high linoleic acid diet being ineffective in reducing blood pressure or attenuating the development of hypertension, renal prostaglandin synthesis of the two series was similar in rats fed a high linoleic acid containing diet and rats fed a diet containing reduced linoleate (Tribe et al. 1976a; Codde 1985). Similarly, in 2-kidney 1-clip renal models, no significant change in renal or aortic prostaglandin production was shown to be associated with an increased dietary intake of linoleic acid despite a highly significant change in aortic and renal phospholipid fatty acid complement, which included a dramatic
increase in lipid linoleate (Codde et al. 1984). Therefore, it appears that in rats, whether the hypertension is genetic in origin or experimentally induced, dietary linoleate supplementation which significantly increases tissue n-6 PUFA content, is ineffective in preventing the rise in blood pressure.

There is evidence to suggest that the amount of dietary fat may be important in blood pressure regulation. The blood pressure in Sprague-Dawley rats following a nine week feeding programme of 5% fat diet containing 2.4% linoleic acid was lower than that of rats fed 20% fat diet containing 9.6% linoleic acid (Smith-Barbaro et al. 1981). In addition Wexler (1981) prevented the development of hypertension in the SHR by feeding 5 week old animals a 29% fat diet containing 0.25% linoleic acid for 12 weeks. Although this diet was apparently atherogenic, these and other studies suggest that the blood pressure regulating effect of dietary fat in rats may depend on the level as well as the type of fat (Hoffman et al. 1982; Smith-Barbaro et al. 1981), and the results obtained may also depend upon the model of hypertension studied.

**THE ROLE OF DIETARY FAT IN BLOOD PRESSURE REGULATION**

Membrane lipid fatty acid profiles in blood cells have been shown to be different in vegetarians compared with omnivores (Sanders et al. 1978). A major difference in the proportion of n-6 PUFA present in erythrocyte membranes of vegetarian subjects may modify membrane fluidity, and this has been shown to influence a variety of functions of blood cells and other tissues including surface receptor coupling (Orly and Schramm 1975), cell proliferation (Corps et al. 1980), platelet
aggregation (Lorenz et al. 1983), and sodium/potassium-ATPase activity (Dean and Suarez 1981). It is possible that a small change in membrane fluidity of endothelial and smooth muscle cells in blood vessels may precipitate alterations in arterial pressure homeostatic regulatory mechanisms. Several studies carried out in normotensive rats have produced consistent data; diets containing high levels of polyunsaturated fat induce a hypotensive effect, whereas high levels of saturated fat elevate blood pressure (Smith-Barbaro and Pucak, 1983). One criticism of some studies has been that in altering the unsaturation index of a diet the carbon chain length of fatty acids present have been poorly controlled. Furthermore, when the chain length is controlled; the degree of saturation appears to be a key factor in the observed effect of the dietary intervention. Although an increased dietary intake of polyunsaturated fatty acids may change cell membrane fluidity, such a dietary change might be expected to influence also tissue prostanoid metabolism and activity.

In neurogenic and renal models of hypertension in the rat, an increase in dietary linoleate leading to an increase in tissue phospholipid linoleate may not be enough to offset the potent neurohumoral pressor mechanisms in operation. Hypertension in genetically-prone rats is likely to be of more interest as it most closely resembles essential hypertension in man, although there are limitations of extrapolation in much higher animals. In the SHR administration of diets rich in linoleic acid that are capable of reducing blood pressure in normotensive and salt loaded rats appear ineffective. No data are available as yet regarding the incorporation of dietary fatty acids into SHR
tissue phospholipid with respect to subsequent renal and vascular prostaglandin formation. It is established that these rats have an enhanced ability to convert exogenous arachidonic acid into vasodilator prostanoids (Limas and Limas 1977; Pace-Asciak et al. 1978) and it has been suggested that a defective release of arachidonic acid from membranes results in decreased vasodilator prostaglandin production leading to hypertension (Dusting et al. 1981; Dickens et al. 1982) in these animals. An abnormality in tissue phospholipid composition as inferred by observations of a generalised decrease in SHR plasma membrane fluidity (Postnov et al. 1981; Aragon et al. 1982; Orlov et al. 1982) may not be reversible in these rats by dietary means, and this may suggest an abnormality in the metabolic handling of dietary lipid such as that recently reported in human hypertension (Singer et al. 1984).

The mobility and conformation of membrane proteins may be modulated by the physical conditions imposed by the surrounding phospholipids and much interest and speculation has been stimulated by recent reports of abnormal cell plasma membrane structure and function in hypertension. Abnormalities in hypertension are shared by tissues such as adipocytes, blood and nerve cells, and are diverse enough to suggest that the most likely explanation to unite such findings is a genetic disturbance of membrane structure influencing its physical characteristics (Bing et al. 1986). Studies of membrane structure are somewhat less precise than those of ion fluxes (Swales, 1982; Heagerty et al. 1986) focussing upon membrane phase transition temperature and viscosity. Disturbances in erythrocyte membrane fluidity (Orlov and Postnov, 1982) and the phase transition
temperature of lithium efflux (Levy et al. 1983) may indicate a disturbance in the fatty component of membrane phospholipids in hypertension. The resulting abnormal membrane may alter cellular responses to normal levels of circulating vasoconstrictor hormones such as noradrenaline, angiotensin II and vasopressin. The way in which many cells respond to these agonists is only now being evaluated and it appears that plasma membrane bound phospholipids act as mediators between the stimulus and cellular ion transport mechanisms.

THE INOSITOL-CONTAINING PHOSPHOLIPIDS

The inositol lipids appear to be present in all eukaryotic cell membranes and are located predominantly in the inner lamella of the plasma membrane (Abdel-Latif 1986), the exact amounts present probably vary between tissues but it is estimated to be between 0.5 and 2.5 umol per gram of tissue representing approximately 5 to 15 percent of the total membrane phospholipid content (Eisenberg and Hauser 1969). The major proportion of this lipid is phosphatidylinositol (Ptd-Ins), with trace contributions from two others, phosphatidylinositol 4-phosphate (Ptd-Ins 4P) and phosphatidylinositol 4,5-bisphosphate (Ptd-Ins 4,5 P$_2$) (Hawthorne and Michell 1966). The polyphosphoinositides are formed by specific membrane bound kinases, phosphatidylinositol kinase phosphorylates Ptd-Ins in the 4 position thereby producing Ptd-Ins 4P which is the precursor to Ptd-Ins 4,5 P$_2$ formed by subsequent phosphorylation in the 5 position catalysed by phosphatidylinositol 4-phosphate kinase (Abdel-Latif 1986). Two polyphosphoinositide monoesterases are also present in membranes which are able to dephosphorylate Ptd-Ins 4P and Ptd-Ins 4,5 P$_2$.
so that in the absence of a cellular agonist these inositol lipids are constantly undergoing metabolic turnover in a so called futile cycle (Berridge and Irvine 1984). The existence of these three lipids was described nearly thirty years ago (Paulus and Kennedy 1960; Grado and Ballou 1961) and there is little doubt that other structurally similar compounds may exist whose functions as yet remain to be characterised (Klenk and Hendricks 1961; Ellis et al. 1963; Tysnes et al. 1985; Saltiel et al. 1986).

Polyphosphoinositides have a high binding capacity for divalent cations including calcium (Buckley and Hawthorne 1972) and it is believed that the interconversion of Ptd-Ins 4P and Ptd-Ins 4,5 P$_2$ may play an important role in calcium binding to the inside of the plasma membrane. If the production of Ptd-Ins 4,5 P$_2$ is favoured to its dephosphorylation then this may facilitate the binding of a higher proportion of intracellular cation to the polar phosphate groups of Ptd-Ins 4,5 P$_2$ (Henrickson and Reinertsen 1969).

**INTRACELLULAR SIGNALLING AND PHOSPHOINOSITIDES**

Enzymic activity within the phosphoinositide system can be triggered by a number of different agonists or hormones that evoke a response within cells by modifying the intracellular free ionised calcium concentration. The binding of these agents to specific receptor proteins located facing the outer cell surface and subsequent interaction of this agonist-receptor complex with a novel guanine nucleotide binding protein (G-protein) (Cockcroft and Gomperts 1985; Michell and Kirk 1986) breaks the membrane phosphoinositide futile cycle by activating a specific
phospholipase C (PLC) which hydrolyses Ptd-Ins 4,5 P2 and releases two compounds with different second messenger functions. The water soluble head group, inositol 1,4,5-trisphosphate (Ins 1,4,5 P3) is released into the cytoplasm and the hydrophobic product 1,2-diacylglycerol (1,2-DG) is retained in the membrane (Berridge 1986).

**INOSITOL PHOSPHATES**

Ins 1,4,5 P_3 has been shown to release calcium from non-mitochondrial organelles (Streb et al. 1983; Prentki et al. 1985) by way of a guanine nucleotide regulatory mechanism (Gill et al. 1986). In smooth muscle cells this triggers contraction (Somlyo et al. 1985), and in other tissues the release of intracellular calcium initiates the prime step in eliciting the physiological response (Abdel-Latif 1986; Berridge and Irvine 1984). This messenger molecule can be deactivated in two ways, it may undergo 5'-dephosphorylation to inositol 1,4-bisphosphate (Ins 1,4 P_2), which does not appear to release intracellular calcium itself (Berridge and Irvine 1984) but can be further metabolised to inositol 1- and 4- monophosphates and thence to inositol (Storey et al.1984). The second route of Ins 1,4,5 P_3 removal requires phosphorylation by a specific Ins 1,4,5 P_3 -kinase producing inositol 1,3,4,5-tetrakisphosphate (Ins 1,3,4,5 P_4) (Batty et al. 1985). This larger molecule may be dephosphorylated by the 5'-phosphatase which deactivates Ins 1,4,5 P_3 to yield inositol 1,3,4-trisphosphate (Ins 1,3,4 P_3) (Michell and Kirk 1986). Further dephosphorylation of this trisphosphate isomer to inositol has yet to be elucidated, and possible messenger functions of Ins 1,3,4,5 P_4 metabolites remain unknown. Recent
work in sea urchin eggs suggests that Ins 1,3,4,5 P₄ itself has a role in regulating the entry of calcium ions into cells, possibly to replenish stores depleted by its precursor (Irvine and Moor 1986). Also Ins 1,3,4,5 P₄ formation is stimulated by a rise in intracellular calcium such as that mediated by Ins 1,4,5 P₃ (Irvine and Moor 1986). The generation of inositol phosphates in response to agonist occupation of receptors is rapid; where it has been investigated, Ins 1,4,5 P₃ reaches its maximal concentration within the first five seconds of stimulation and can mediate the release of stored calcium and may control calcium flux.

In some circumstances PLC activation may release a cyclic form of Ins 1,4,5 P₃, inositol 1:2 cyclic 4,5-trisphosphate (Ins 1:2 cyclic 4,5 P₃) (Berridge 1986; Majerus et al. 1986; Williamson 1986) rather than the non-cyclic form. This appears to be pH dependent (Berridge 1986) as it seems that both phosphates are formed in many tissues with the major proportion being the non-cyclic form unless the pH becomes very acidic (Majerus et al. 1986). It is becoming increasingly evident that Ins 1:2 cyclic 4,5 P₃ has functions of its own being able to release calcium in blood platelets and pituitary cells, and to stimulate photoreceptors in the horse shoe crab (Wilson et al. 1985). The less polar dephosphorylated cyclic phosphates await precise functional characterisation. If PLC mediated cleavage of Ptd-Ins 4,5 P₂ is the primary response to agonist-evoked stimulation, detailed studies in smooth muscle and other cells now suggest that subsequently PLC activity is directed against Ptd-Ins 4P and Ptd-Ins (Griendling et al. 1986; Imai and Gershengorn 1986; Majerus et al. 1986). In this way all three characterised
phosphoinositides can be hydrolysed if receptor stimulation is continued allowing the production of the other messenger molecule, 1,2-DG to proceed at a rate in excess of that liberated from Ptd-Ins 4,5 P$_2$. This is most probably the key to sustained agonist induced physiological responses in cells. PLC may attack Ptd-Ins 4,5 P$_2$ initially, other phosphoinositides having a decreased affinity for the phospholipase or may require a small rise in cellular calcium prior to enzyme activation. Alternatively different subtypes of PLC may exist being activated in sequential fashion. Recent work suggests at least two such enzymes are present (Ryu et al. 1986).

1,2-DIACYLGLYCEROL

1,2-diacylglycerol has a second messenger role in the cell membrane where it activates protein kinase C (Nishizuka 1986). This enzyme is found in all tissues and requires calcium, phosphatidylserine and diacylglycerol for activation (Hirasawa and Nishizuka 1985; Nishizuka 1984). The affinity of protein kinase C for calcium is increased by 1,2-DG and this appears to be dependent upon the chain length and degree of unsaturation of fatty acyl groups esterified in the original phosphoinositide (Kishimoto et al. 1980; Kaibuchi et al. 1981). Naturally occurring diacylglycerols have extremely short half lives, either being hydrolysed to release monoacylglycerols and fatty acids or phosphorylated to phosphatidic acid. Therefore most of the information regarding the actions of this second messenger has been obtained using non-hydrolysable analogues of 1,2-DG such as the tumour-promoting phorbol esters. These compounds can activate protein kinase C. However the use of phorbol esters has been
criticised as they are not susceptible to enzyme degradation. Nevertheless, such compounds are useful tools in clarifying the functions of 1,2-DG mediated activation of protein kinase C which normally occurs in synergism with Ins 1,4,5 P$_3$-induced intracellular calcium release.

Protein kinase C controls the influx of calcium through voltage sensitive calcium channels (Wakade 1986) and the influx of sodium through the activation of the sodium proton exchange system, there is also evidence to suggest that protein kinase C can influence the efflux of calcium (Drummond 1985; Rickard and Sheterlin 1985) and sodium (Greene and Lattimer 1986; Simmons et al 1986) through activation of their respective ATPases. An increasing number of reports are now accumulating which suggest a negative feedback mechanism on agonist-induced stimulation of PLC as a role for protein kinase C. Brock et al. (1985) have reported that the synthetic diacylglycerol 1-oleoyl 2-acetylglycerol and the phorbol ester 4-phorbol 12-myristate 13-acetate can inhibit angiotensin II stimulation of PLC. This action would appear to be mediated through the uncoupling of the agonist-receptor-G-protein complex from PLC induced by protein kinase C activation, and may well be the mechanism by which hormones such as angiotensin II induce tachyphylaxis when applied to vascular tissue (Brock et al. 1985). Similarly, further evidence suggests this phenomenon is also seen with adrenergic agonists and smooth muscle cells (Colucci et al. 1986).

**ARACHIDONIC ACID**

Inositol phospholipids are highly enriched with arachidonic acid at the sn-2 position of the glycerol component (Holub 1982).
An increase in the release of arachidonic acid and the production of dienoic prostaglandins has been demonstrated following agonist-evoked hydrolysis of phosphoinositides presumably via diglycerol lipase activity on liberated 1,2-DG (Coughlin et al. 1984; Rittenhouse 1984). In phagocytic cells and macrophages there is evidence that arachidonic acid itself can initiate physiological functions (Badway et al. 1981; Walsh et al. 1981) and can activate protein kinase C (McPhail et al. 1984). Therefore this fatty acid may have important messenger functions of its own in receptor function and cellular regulation in addition to providing the precursor for prostanoid synthesis whose role at a cellular level are of undoubted significance (see figure 3).

SMOOTH MUSCLE CONTRACTION

Through such membrane and cytosolic messenger systems investigators are beginning to appreciate how smooth muscle cell contraction is triggered, maintained and modulated. There is little doubt that once a vasoconstrictor agonist occupies its receptor Ins 1,4,5 P_3 is released into the cytoplasm and stimulates the release of calcium from a nucleotide dependent pool in the sarcoplasmic reticulum in smooth muscle (Abdel-Latif 1986). The resulting increase in intracellular calcium may be potentiated by the release of calcium bound to the inside of the plasma membrane, another function influenced by phosphoinositide hydrolysis (Buckley and Hawthorne 1972). Inositol 1,4,5-trisphosphate mediated calcium release is detectable within 5 to 10 seconds of receptor activation (Burgess et al. 1984) and is of sufficient magnitude to initiate tension development (Snematsu
via calcium-calmodulin mediated activation of myosin light chain kinase (Van Breemen 1986). The precise mechanism by which a contraction is maintained is unclear, however, fortification must rely upon synergy between Ins 1,4,5 P_3 and 1,2-DG/protein kinase C activation. Indeed, phorbol esters have been shown to induce slow developing and sustained contractions in arterial strips (Itoh and Lederis 1987) and these can be blocked by verapamil (Turla and Webb 1986). Thus calcium influx is stimulated through calcium channels. The entry of calcium is partially voltage dependent and therefore related to transmembrane sodium flux which is prompted by activation of the sodium/proton exchange system (Moolenaar et al. 1982), the importance of which in vascular smooth muscle cells has recently been reported (Little et al. 1986). Indeed, the link between cellular calcium metabolism and this antiport is now firmly established. Siffert and Akkerman (1987) have demonstrated that inhibition of the sodium/proton exchange mechanism with amiloride abolishes the transient rise in cytosolic calcium usually observed in thrombin stimulated platelets. More relevant was the finding that repeating the experiments with ethylisopropylamiloride, a more specific inhibitor of the antiport, produced similar results. In addition, the functional properties of platelets were profoundly altered, with decreased aggregability and reduced ability to change shape. It now appears that activation of cultured vascular smooth muscle cells with vasoconstrictor agonists such as noradrenaline and angiotensin II leads to increased sodium/proton exchange (Owen 1986; Berk et al. 1987) and Haddy et al. (1985) have reported that in vivo amiloride and its analogues induce vasodilatation in the dog. Recent work suggests the pretreatment
of intact arterioles with amiloride or ethylisopropylamiloride in vitro will prevent sustained agonist evoked contractions (Bund et al. 1987). Therefore this antiport assumes a central role in the preservation of cellular functional integrity.

**SMOOTH MUSCLE RELAXATION**

If pressor agents effect constriction of vascular tissue by stimulating inositol phosphate and diacylglycerol production, the mechanism for relaxation is only recently being unravelled. Many vasodilators such as sodium nitroprusside, nitrates and phenylhydrazine, raise cellular cyclic guanosine monophosphate (cGMP) (Rapoport and Murad 1983) and reports now indicate that this compound may exert its actions via inhibition of phosphoinositide hydrolysis (Rapoport 1986). Indeed, Lockette and Otsuka (1986) were unable to relax smooth muscle contracted with the phorbol ester 12-0-tetradecanoylphorbol-12-acetate using 8-bromo-cGMP, a cell permeable analogue of cGMP, but this contraction would have been elicited without recourse to triggering the phosphoinositide cascade. Acetylcholine also raises smooth muscle concentrations of cGMP, blood vessels relax and phosphoinositide hydrolysis is inhibited in the presence but not the absence of endothelium (Rapoport 1986), indicating a role for endothelium derived relaxing factor in this function. Therefore it is possible that an equilibrium is present between vasodilators and relaxation mechanisms such as those mediated through inhibition of phosphoinositide hydrolysis on the one hand, and vasoconstrictor agonists and the phosphoinositide system on the other. Thus in cultured aortic smooth muscle cells it has been shown that vasopressin induces contraction by
stimulation of phosphoinositide hydrolysis and subsequent calcium influx as well as inhibiting B-adrenergic cyclic adenosine monophosphate accumulation and also atrial natriuretic peptide induced cGMP accumulation (Nambi et al. 1986). The contributions from both positive and negative systems will determine the degree of smooth muscle cell activation elicited by an agonist, and a derangement in either may contribute to some of the phenomena observed in hypertension.

**CELL GROWTH AND PROLIFERATION**

It has been known for some time that the application of growth factors to quiescent cells in culture causes a rapid increase in sodium influx and subsequent activation of the sodium pump (Rozengurt 1981). Sodium influx here probably occurs via the sodium/proton exchange system as the characteristic sodium influx and mitogenic properties of compounds such as platelet derived growth factor can be blocked with amiloride. Activation of the sodium/proton exchange system leads to the alkalinisation of the cell cytosol (Burns and Rozengurt 1983; Rothenberg et al. 1983; Berk et al. 1987) and this appears to be the cellular signal for deoxyribose nucleic acid (DNA) synthesis and protein translation (Rozengurt 1986; Vincentini and Villereal 1986). In this regard amiloride has been shown to prevent cellular proliferation (L'Allemain et al. 1984). It is well established that growth factors stimulate phosphoinositide hydrolysis (Vincentini and Villereal 1986) and the sodium/proton exchanger which can be activated by phorbol esters (Dicker and Rozengurt 1981, Moolenaar et al. 1984) but is normally controlled by 1,2-DG/protein kinase
C activation (Nishizuka 1984). This, together with accompanying rapidly stimulated calcium efflux (Lopes-Rivas and Rozengurt 1983) form part of changes in ion handling which are initiated within seconds of the initial stimulus, the actual onset of DNA synthesis is likely to be some hours later and it is probable that these early changes are merely factors in a carefully orchestrated response to the influx of sodium. In addition, recently reports have linked certain oncogene products with a deranged phosphoinositide system (Marcana 1985) and have stimulated speculation that abnormalities in this signalling system may be implicated in carcinogenicity. Similarly, as a number of workers are now beginning to realise, in a disease of altered growth in vascular tissue such as hypertension, the phosphoinositide signalling system may be of great interest as abnormalities in this system could account for many of the observed abnormalities in cation handling and structural changes of the vasculature found to be associated with hypertension (see figure 4).

PHOSPHOINOSITIDE SIGNALLING IN HYPERTENSION

Research into the phosphoinositide signalling system in hypertension for the most part has centred around the measurement of the incorporation of the radiolabelled terminal phosphate of \[^{32}\text{P}\] adenosine triphosphate into the polyphosphoinositides, and studies have utilised membrane fractions prepared from circulating blood cells taken from genetically hypertension prone animals and hypertensive patients. In erythrocyte membranes from human hypertensive patients, available information suggests that the Ptd-Ins content is normal
or slightly reduced (Minenko et al. 1981), whereas in SHRs the converse is true (Boriskina et al. 1978; Kiselev et al. 1981; Koutouzov et al. 1981). The rate of incorporation of $^{32}$P into the polyphosphoinositides in hypertensive man appears not to be different from control subjects when applying analysis of variance (Marche et al. 1985) and the picture is far from clear in erythrocyte membranes prepared in the adult SHR with established hypertension, phosphorylation of phosphoinositides may also vary with age (Kiselev et al. 1981). Many of the defects in cation transport have been identified in erythrocytes taken from both hypertensive patients and their offspring before blood pressure had risen (Swales 1982; Heagerty et al. 1986), an association between these phenomena and the phosphoinositide signalling system would seem attractive, but sodium and calcium perturbations are found in established hypertension where in humans phosphoinositide metabolism is apparently normal, if the postulated association is real then either the relation is lost or confounded by the disease process itself.

In common with other prokaryotic cell types, erythrocytes are devoid of many metabolic pathways that are present in eukaryotes including portions of the phosphoinositide signalling system (Downes and Michell 1981). Rather than calcium release from internal stores, the phosphoinositides control cell shape in erythrocytes (Allan and Michell 1979; Downes and Michell 1981) and in this regard erythrocyte filterability is reduced in hypertensive patients (Zannad et al. 1985). The measurement of $[^{32}$P] labelling of polyphosphoinositides is an index of synthesis of these lipids, constant hydrolysis and dephosphorylation of the polyphosphoinositides makes interpretation of $[^{32}$P] incorporation
difficult in such studies. In addition, the paucity of red cell surface receptors make it a poor model for studies of agonist stimulation where an analysis of chain length and degree of unsaturation of the acyl chains esterified within the released diglyceride may underly a functional difference of activating the system in hypertension. Recently however, in erythrocytes taken from SHRs Kato and Takenawa (1987) have observed an increase in Ptd-Ins phosphorylation which appears to be coupled with an enhanced PLC activity, and Postnov et al. (1987) have revealed a possible functional abnormality in protein kinase C activation using the phorbol ester 4B-phorbol 12 B-myristate 13-acetate.

An alternative approach in studies of phosphoinositide metabolism is to introduce \(^{3}\text{H}\)-myoinositol as the precursor to phosphoinositide synthesis in the presence of lithium. Lithium ions prevent the enzymatic degradation of inositol monophosphate to inositol and therefore facilitates the rate of water soluble inositol phosphate accumulation to be examined in both the basal and the agonist-stimulated state (Berridge et al. 1982). This technique has been applied to vascular tissue (Fox et al. 1985; Rapoport 1986) and cells cultured from vascular and neuronal tissue taken from young and adult SHRs and control rats where differences in inositol phosphate accumulation rates were found only in neuronal tissue cultured from young SHRs (Nabika et al. 1985; Feldstein et al. 1986). However, cell culture requires the administration of antibiotics to the growth medium and commonly used antibiotics have been shown to inhibit hydrolysis of Ptd-Ins \(4,5\ P_2\) (Marche et al. 1987), in addition after several generations of cultured smooth muscle cells contractile protein and calcium channels are gradually lost (Chamley-Campbell et al.
presumably as a consequence of this, contractions in these cells can no longer be demonstrated (Chamley-Campbell et al. 1979) and their value as a reflection of the original tissue is questionable. Although as yet no study has examined vascular phosphoinositide signalling in hypertension, potentially providing a mechanism through which structural changes in blood vessel media could be effected. Recent studies in SHR have shown enhanced sodium/proton exchange in thymocytes (Feig et al. 1987) and in vascular smooth muscle cells in culture (Kuriyama and Aviv 1986). Also, changes in plasma bicarbonate and ultimately blood pH have been demonstrated in these rats (Lucas et al. 1987; Izzard and Heagerty 1988). Elaborate work by Bevan (1984) suggests that as well as circulating hormones, sympathetic nervous system tone also determines vascular structure. Sympathetic trophic effects upon muscle bulk, extracellular artery components and vessel number were seen during maturation of rats or rabbits (Bevan and Tsuru 1981) and agents such as noradrenaline, angiotensin II and vasopressin which cause vaso-constriction can also induce proliferation of smooth muscle cells in culture (Campbell-Boswell and Robertson 1981; Blaes and Boissel 1983). This trophic action is probably attributable to 1,2-DAG/kinase-C activation. It is conceivable therefore for a variety of stimuli to proliferate medial muscle during the genesis of hypertension via activation of phosphoinositide hydrolysis.
In summary, there is a large volume of evidence now to suggest that genetic modifications of the physicochemical structure of the plasma membrane may be crucial to the development of raised pressure in human essential hypertension and in genetically hypertension-prone rats. Both hydrophilic and lipophilic components of membrane phospholipids may play a fundamental role in the regulation of growth and vascular reactivity in the myocytes of peripheral arteries. A formal assessment of plasma membrane lipid composition has not been undertaken: therefore, my first experiments were carried out to measure the membrane fatty acid content in essential hypertension and in the SHR with appropriate normotensive controls for comparison.

In subsequent experiments I have carried out dietetic manipulations of lipid intake in order to investigate whether changing the membrane environment could alter electrolyte transfer and blood pressure; these studies were executed both in man and in genetically hypertension-prone rats. Finally, in the light of the possible implications of complex phospholipids in the control of growth and vascular tone, I have extended my experiments to examine phosphoinositide lipid hydrolysis in hypertension, both in human erythrocytes, vascular tissues from the SHR, and during the induction of aortic coarctation. The object of these experiments is to further our knowledge about cell membrane lipid composition and metabolism in hypertension: it is my hope that the studies will provide more information about the cellular mechanisms at work in this disease and my results are described below.
Fig 1. Structural diagram showing chemical composition of two polyunsaturated fatty acids
<table>
<thead>
<tr>
<th>n-6 series</th>
<th>prostaclan</th>
<th>n-3 series</th>
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<tbody>
<tr>
<td>18:2 linoleic</td>
<td></td>
<td>18:3 α-linolenic</td>
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<tr>
<td></td>
<td>18:3 γ-linolenic</td>
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<td></td>
<td>20:3 dihomo-γ-linolenic</td>
<td>20:5 eicosapentanoic</td>
</tr>
<tr>
<td></td>
<td>20:4 arachidonic</td>
<td>22:6 docosahexaenoic</td>
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Figure 2. Polyunsaturated fatty acid metabolism and prostaglandin synthesis.
Figure 3. Schematic illustration of the major phosphoinositide lipids and their products of hydrolysis. AII, Angiotensin II; NA, noradrenaline; ADH, antidiuretic hormone; PLC, phospholipase C; CDP-DG, cytidine diphosphate diglyceride; ER, endoplasmic reticulum; cyclic IP, cyclic inositol phosphate; for other abbreviations see text.
Figure 4. Cellular functions mediated or influenced by phosphoinositide lipids in smooth muscle. EDRF, endothelial-derived relaxing factor; ANP, atrial natriuretic peptide; other abbreviations as in text.
CHAPTER 2

BIOCHEMICAL AND EXPERIMENTAL METHODS
2.1 BIOCHEMICAL METHODS

ERYTHROCYTE LIPID FATTY ACID ANALYSIS

Erythrocyte lipid fatty acid analyses were performed on 10ml samples of non-fasting blood taken from the antecubital vein. Red cells were separated from white cells and platelets by centrifugation at 4°C (15 minutes, 2000g); cells were then resuspended and washed three times in ten volumes of cold normal saline by centrifugation at 4°C (12 minutes, 2000g). One millilitre (ml) aliquots of packed cells were lysed in an equal volume of distilled water; this was done in duplicate in two 20ml glass centrifuge tubes. Lipid extractions were performed according to the method of Rose and Ocklander (1965); 11mls of propan-2-ol containing 0.45mmol butylated hydroxytoluene as an antioxidant was added and the tubes were then agitated and allowed to stand on ice for 1 hour. Seven mls of chloroform were then added and the tubes again agitated and left to stand for a further hour prior to sedimentation of tissue debris by centriguation at 4°C at 600g for 10 minutes. The lipid-containing solvent was then decanted into glass universal tubes and stored at -20°C under an atmosphere of nitrogen.

Transesterification of lipid to fatty acid methyl esters was carried out according to the method of Christie (1972); lipid extracts were taken to dryness in a waterbath at 60°C under a stream of oxygen-free nitrogen. 300 microlitres (ul) of hexane and 100ul sodium methoxide solution (200umol in methanol) were sequentially added to the dried extract, the tubes were capped and allowed to mix for five minutes on a Spiramix rotary mixer (Denby, Sussex, UK). 2.5ml hexane and 0.5grams (gm) granular calcium chloride were added, the tubes capped and the contents
were mixed and allowed to stand at room temperature for 60 minutes. After this time the tube contents were filtered through a Whatman number 1 filter disk, premoistened with hexane, into 5ml capacity glass scintillation vials. The residue left in the universal tube was washed with 2ml hexane and filtered. Filtrates were pooled and taken to dryness under oxygen free nitrogen in a water bath at 60°C. The dried methyl esters were then reconstituted in 200μl hexane. A one to two microlitre aliquot of each sample was injected into a Perkin Elmer F17 gas liquid chromatograph fitted with a flame ionisation detector and a 2 metre 15% diethylene glycol succinate (DECS) on Chromosorb W (100-200 mesh) column (Perkin Elmer, Buckinghamshire, UK) running isothermically at 180°C with nitrogen as the carrier gas at a flow rate of 50ml per minute, the injector/detector block temperature was 250°C. Fatty acid methyl esters were identified by comparing retention times with those of authentic standards (obtained from Sigma Chemicals, Dorset, UK). Peak heights were measured electronically using a Schimadzu Chromatopac Integrator (Dyson Instruments Ltd, Tyne and Wear, UK). Individual fatty acid methyl esters were expressed as a percentage relative to all fatty acid methyl esters chromatographed. The above chromatographic conditions were optimised through repeated injections and chromatography of authentic fatty acid methyl ester mixtures (Sigma Chemical, Dorset, UK) and facilitated routine resolution of derivatives corresponding to five major fatty acids found in biological membranes, these were palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 n⁻⁹), linoleic acid (18:2 n⁻⁶) and arachidonic acid (20:4 n⁻⁶). The coefficients of variation for erythrocyte lipid fatty acid
estimations were calculated as follows: 16:0 0.05%, 18:0 0.05%,
18:1 n-9 0.05%, 18:2 n-6 0.08%, 20:4 n-6 0.09%.

ERYTHROCYTE PHOSPHOINOSITIDE METABOLISM

On the day of study 10ml freshly drawn peripheral venous
blood was centrifuged at 2000g (4°C) for 15 minutes, the plasma
and buffy coat were removed by aspiration using transfer plastic
pipettes (Sarstedt, Rommelsdorf, West Germany). The erythrocytes
were then washed three times with 0.9% sodium chloride solution,
and membranes were prepared according to the method of Schneider
and Kirschner (1970); cells were lysed at 4°C in 1.44mM Tris-
HCl/1mM potassium diaminoethantetra acetic acid(EDTA)/17mM NaCl,
(pH 7.5), and centrifuged at 13,000g for 10 minutes in 20mM Tris-
HCl, (pH 7.5). Centrifugations were repeated five times until a
suspension of white membranes was obtained; this was stored at -
70°C and was used within 48 hours.

Incorporation of $^{32}$P into membrane lipids was performed in
duplicate using the method of Marche et al. (1982). Having
estimated the protein content of the membrane suspension by the
method of Lowry et al. (1951), 0.3mg of membrane protein was
taken and diluted to 200ul with 20mM Tris-HCl, 200ul of 80mM
Tris-HCl was then added to give a final volume of 400ul of 50mM Tris-
HCl, pH 7.5. The membranes were then pre-incubated for ten
minutes at 37°C, and 100ul of 10mM [$^{32}$P]-ATP (specific activity
30-50uCi.mmol$^{-1}$) and 25mM MgCl$_2$ prepared in 50mM Tris-HCl were
added to initiate phosphorylation at a final concentration of 5mM
[$^{32}$P]-ATP and 5mM MgCl$_2$. In order to obtain the required specific
activity of [$^{32}$P]-ATP,3-5uCi (1.7nmols) of radiolabelled ATP
(specific activity 2000-3000Ci.mmol$^{-1}$ Amersham, Buckinghamshire,
UK) was added to a 10mM solution of non-radioactive ATP. The membranes were incubated for 15 minutes in a shaking water bath at 37°C and the reaction was stopped by the addition of chloroform/methanol/HC1 specific gravity 1.18 (20/40/1 V/V/V) directly to the incubation medium. The suspension was left for 10 minutes to allow extraction of lipid, and then the single phase was partitioned by the addition of 3ml of chloroform and 3ml of water. The resulting upper phase was removed together with interstitial precipitated protein, and the remaining solvent phase was evaporated to dryness under nitrogen at room temperature, dissolved in 25ul of chloroform and stored at -20°C overnight.

Phospholipids were separated by thin layer chromatography. Individual 5716 cellulose thin layer chromatography plates (Merk, Darmstadt, West Germany) were heated to 110°C for 20 minutes in an oven and left to cool, in order to activate the plate and evaporate the moisture. Each duplicate sample was spiked with 5ul of inositol phospholipids (Ptd-Ins,Ptd-Ins 4P,Ptd-Ins 4,5 P_2, Sigma Chemicals, Dorset, UK), at 20mg.ml^-1 chloroform, this would enable visualisation of[32P]-labelled phosphoinositides following chromatography. Samples were added to the plate using a 30ul capillary tube as a 2-3cm line which was quickly dried using a chromatography plate air dryer. Plates were developed in a monophasic solvent system of butan-1-ol/glacial acetic acid/water (75/10/25 V/V/V) for two hours and dried in air, the lipids were stained by immersing plates in a 27uM solution of Nile blue in 0.1N sulphuric acid. Individual phosphoinositides were identified by comparison of Rf values obtained using authentic standards. The regions corresponding to Ptd-Ins 4P (Rf = 0.26) and Ptd-Ins
4,5-P₂ (RF = 0.19) were scraped off and collected in 5ml scintillation vials (Sterilin, Middlesex, UK). 4ml of OptiPhase 'X' scintillant (LKB, Surrey, UK) was then added before counting for radioactivity in a Tricarb liquid scintillation counter (Canberra Packard, Berkshire, UK).

The mean of duplicate samples was calculated for Ptd-Ins 4P and Ptd Ins 4,5-P₂ as these have been previously shown to be the only phosphoinositides to incorporate radiolabelled ATP (Koutouzov et al. 1982). Also as indicated by Palmer (1985) [³²P]-ATP incorporation into these lipids in erythrocytes proceeds at a constant rate irrespective of cell age.

VASCULAR PHOSPHOINOSITIDE METABOLISM: ANALYSIS BY PHOSPHATE ACCUMULATION

The rate of phosphoinositide hydrolysis in vascular tissue was investigated following the 'batch method' of inositol phosphate analysis described by Berridge et al. (1982) with slight modifications. Aortae were taken from freshly killed rats and placed in tissue culture medium (M199, Gibco, Paisley, UK) containing (mM): NaCl 137, KCl 5.4, MgSO₄·7H₂O 0.81, Na₂HPO₄·7H₂O 0.36, CaCl₂ 1.26, NaHCO₃ 4.2, KH₂PO₄ 0.44, Fe(NO₃)₃·9H₂O 0.002. Each aorta was dissected free of fat using a Technival II stereo dissecting microscope (Carl Zeiss, Jena, West Germany) and a KL 1500 fibreoptic light source (Schott, Wiesbaden, West Germany) and allowed to equilibrate at 37°C for 30 minutes. 200pmols [³H]-D- myoinositol (specific activity 10-20 Ci:mmol⁻¹, New England Nuclear, Boston, MA, USA) in ethanol/water (9/1 V/V) was pipetted into a 1.5ml capacity plastic ependorf centrifuge tube and evaporated to dryness under a stream of oxygen free nitrogen. The
dry residue was redissolved in 200ul M199 containing 10mM lithium chloride, 4mg.ml⁻¹ bovine serum albumin, 0.11mM ascorbic acid, also noradrenaline and prazosin where indicated. The addition of lithium chloride facilitates the accumulation of inositol phosphates as lithium ions inhibit the dephosphorylation of inositol monophosphate (Berridge et al. 1982). Segments of vascular tissue were placed in separate ependorf tubes at the start of the incubation which proceeded for 120 minutes in a shaking water bath at 37°C. After this period the tissue was removed from the incubation medium, blotted dry on a filter disk and weighed before homogenisation in 0.5ml chloroform/methanol/HCl specific gravity 1.18 (20/40/1 V/V/V) at 4°C in a 1ml glass/glass tissue grinder (Gallenkamp, Leicestershire, UK). In this way results could be normalised for tissue wet weight. In some experiments data was normalised to tissue protein content and in these cases tissue was not weighed but a protein estimation was carried out subsequently. The homogenate was left on ice for 10 minutes after which time 0.5ml chloroform and 0.5ml water were sequentially added. The tubes were agitated then centrifuged at 2000g in a bench top Micro Centaur centrifuge (MSE, Sussex, UK) to separate the aqueous and organic phases. The upper aqueous phase contained inositol phosphates and was carefully pipetted to a 10ml plastic test tube (Sarstedt, Rommlesdorf, West Germany) and neutralised by the addition of 43ul 2M NaOH. Separation of [³H]-inositol phosphates from [³H]-inositol was achieved using anion exchange chromatography (Berridge et al. 1982). To bind polar inositol phosphate, 0.5ml of Dowex AG1-X8 anion exchange resin in the formate form (BioRad, Hertfordshire, UK) was added as a slurry in water, the tube was agitated and left to stand to allow
the resin to settle. The supernatant was then aspirated and discarded. The resin was washed five times with 5ml 5mM unlabelled myoinositol and subsequently washed twice with 5ml 1M ammonium formate in 0.1M formic acid to elute bound radiolabelled inositol phosphates, and the supernatants were removed and saved. Two ml aliquots of each formate wash were taken in duplicate and added to 12ml OptiPhase 'X' scintillation fluid and radioactivity measured in a Tricarb liquid scintillation counter. The amount of \[^3H\]-inositol phosphate accumulated was expressed as counts per minute (cpm) per mg wet weight of tissue. In some experiments disintegrations per minute (dpm) could be calculated using appropriate quenched radioactive standards and a knowledge of the specific activity of radiolabelled inositol, allowing the \[^3H\]-inositol phosphate accumulated in tissue segments to be expressed as pmol per mg tissue. This calculation in no way implies the actual amount of inositol phosphate is being expressed but only that which is radiolabelled.

In sections of rat aorta the intra assay variation was calculated as 6.6%. The accumulation of \[^3H\]-inositol phosphate in rat aorta was linear over the 120 minutes incubation period in both control and stimulated conditions in the presence of 10mM lithium chloride (Figure 1a). A dose dependent relationship was observed for the inhibitory effect of lithium chloride upon degradation of inositol phosphate (Figure 1b), and when aortic segments were incubated in increasing concentrations of noradrenaline, a dose response curve for agonist induced stimulation of phosphoinositide hydrolysis could be constructed (Figure 2a). This tissue required 3uM and 100uM doses of noradrenaline for 50% and maximal stimulation respectively, was
similar to responses observed by Fox and Friedman (1987) and is believed to represent a receptor reserve phenomenon for accumulation of inositol phosphates (Minneman and Abel, 1984; Lynch et al. 1985). This is because the maximum dose for hydrolysis of phosphoinositides is at concentrations of noradrenaline far above that needed to elicit maximal smooth muscle contraction; therefore it would appear that noradrenaline is more potent in activating vascular contraction than activating phosphoinositide hydrolysis indicating a receptor reserve for α-receptor evoked contraction. Similar confirmatory observations have been also made in rat brain (Kendall et al. 1985). When increasing doses of the antagonist prazosin were added to incubations containing maximal stimulatory doses of noradrenaline, a dose dependent inhibition curve could be constructed (Figure 2b). In aortic segments prazosin was maximally effective at a concentration of 10μM and the concentration for 50% inhibition (ED_{50}) was calculated to be 140nM (Figure 2b). These observations were similar to those made by Kendall et al. (1985) in rat brain and by Fox et al. (1985) in vascular tissue.

VASCULAR PHOSPHOINOSITIDE METABOLISM: ANALYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Four subcutaneous resistance arterioles arising from the axillary artery (length 2-3cm: internal diameter 100-150um) were dissected from two rats, and cleaned of adherent fat and connective tissue using a Technival II stereo dissecting microscope and KL1500 fibreoptic lightsource. Dissecting these small vessels is an intricate procedure and required the use of specialised instruments, these included trabecular scissors (Hans Geuder, Heidelberg, West Germany) and watch-makers forceps.
Following isolation vessels were placed in tissue culture medium (M199) at 37°C and after 30 minutes of equilibration the arterioles were transferred to a tube containing 50ul M199 and 60uM [3H]-myoinositol (specific activity 10-20 Ci.mmol⁻¹), dried and reconstituted as described above. The vessels were left at 37°C for 120 minutes in a shaking water bath to allow incorporation of radio-label into inositol-containing compounds. Following this, 50ul of pre-warmed vehicle (M199) or noradrenaline was added to give a final concentration of 1mM and left for 20 seconds before removal of the tissue for homogenisation on ice in 0.5ml 62mM ice cold trichloroacetic acid in a glass/glass tissue grinder (Gallenkamp, Leicestershire, UK). Noradrenaline was applied at such concentrations to ensure maximal activation of phosphoinositide-linked receptors (Fox and Friedman, 1987).

Following homogenisation, tissue was left on ice for 15 minutes before it was transferred to an ependorf tube for centrifugation for 10 minutes at 2000g in a benchtop Micro Centaur. The supernatant containing the aqueous tissue extract was transferred to a 10ml capacity glass test-tube and washed five times with 10 volumes of water-saturated diethylether (Irvine et al. 1985). The resulting neutralised extract was diluted to 2ml with a solution containing nine nucleotides to assist the identification of radioactive peaks and also served as an indicator of column stability in subsequent chromatography (Irvine et al. 1985; Batty et al. 1985). This standard nucleotide mixture contained cytidine mono- and di-phosphate; adenosine mono-, di- and tri-phosphate; guanosine mono-, di-, tri- and
tetra-phosphate (Sigma Chemicals, Dorset, UK).

Phospholipids were extracted from the sedimented tissue pellet by homogenisation on ice in 0.5ml chloroform/methanol/HCl specific gravity 1.18 (20/40/1 V/V/V). This homogenate was left on ice for 15 minutes before it was transferred to an ependorf tube, then sequential additions of 0.5ml chloroform and 0.5ml water were made to render the extract biphasic. Following agitation and centrifugation at 2000g the upper aqueous layer was discarded and the lower phospholipid-containing organic phase was transferred to a glass test-tube, leaving behind the residual tissue pellet, and evaporated to dryness under a stream of oxygen-free nitrogen (Downes and Wusterman, 1983).

Dried lipid was reconstituted in 1ml of methylamine reagent comprising 25% methylamine solution/methanol/butan-1-ol (4/4/1 V/V/V) and placed in a water bath for 45 minutes at 53°C. After this time the solution was evaporated to dryness and reconstituted in 0.7ml water, the acyl moeties were then removed by washing twice with 0.7ml of a butanol solution which contained butan-1-ol/light petroleum 40'-60'/ethyl formate (20/4/1 V/V/V), the upper phase was discarded and the remaining aqueous phase containing deacylated lipid was diluted to 2ml with a solution containing nine nucleotides prior to chromatography (Hawkins et al., 1986).

Solubilisation of the residual tissue pellet after removal of the lipid-rich organic phase before deacylation was achieved using 0.25ml 2M NaOH, allowing the estimation of protein content by the method of Lowry (1951) and subsequent chromatographic data normalisation with respect to tissue protein content.

In a small number of experiments, glycerol was chemically
removed from deacylated lipid extracts to release $[^3H]-\text{inositol phosphate}$. This was carried out as described by Irvine et al (1985). To 250ul of deacylated lipid extract 12.5ul of 0.2M sodium periodate was added, a head space of nitrogen was given to the tube before it was sealed and placed in the dark at room temperature for 90 minutes. The reaction was quenched with 23.5ul of 1% ethylene glycol solution, the tube was again flushed with nitrogen and left a further 15 minutes at room temperature. To remove the remaining aldehyde group from the inositol 1-phosphate a 1% aqueous solution of 1,1-dimethylhydrazine was taken to pH 4.5 with 2M formic acid and 24.25ul was added to the sample tube. The tube was shaken and after flushing with nitrogen left for four hours at room temperature. The dimethylhydrazine was removed by the addition and mixing of 200ul of Dowex 50W resin slurry (50x4-200, hydrogen form, BioRad, Hertfordshire,UK). The clear supernatant present after the resin had settled was transferred to a clean glass sample tube and diluted with water and nucleotides before chromatography.

Chromatographic separations were performed using a dual pump gradient elution high performance liquid chromatography (h.p.l.c.) system (LKB, Surrey,UK). Aqueous and deacylated resistance arteriole lipid extracts containing the nine marker nucleotides were injected onto a Partisil 10 SAX h.p.l.c. column (250x4.6mm, Technicol, Cheshire, UK) through a Rheodyne sample injector fitted with a 2ml sample loop. The column was eluted at 1.2ml.min$^{-1}$ with a non-linear pre-programmed gradient of water/1.7M ammonium formate/orthophosphoric acid (pH 3.7). On-line ultra violet monitoring at 254nm allowed measurement of retention times and column performance for nucleotide markers and the whole of the
gradient was fractionated into scintillation vials (Irvine et al. 1985; Batty et al. 1985). 1.15ml water and 16ml Ready Safe scintillation fluid (Beckman Instruments, Buckinghamshire, UK) were added to 0.6ml fractions of column eluate before liquid scintillation counting in a Tricarb Counter and quantitation of eluting radioactive peaks as pmol \([^3\text{H}]-\text{inositol containing compound}\) mg protein\(^{-1}\) was achieved using appropriate quenched standards.

**ESTIMATION OF LEUCOCYTE SODIUM TRANSPORT**

The leucocyte sodium transport studies were performed using the method of Milner et al. (1984). Fifty ml of peripheral venous blood was transferred to universal containers, each containing 7.5ml of Plasmagel (Uniscience, Cambridge, UK) and thoroughly mixed. The containers were allowed to stand in a water bath at 37°C which facilitated the sedimentation of erythrocytes. After 30 minutes the supernatant was transferred to plastic 10ml centrifuge tubes (Sarstedt, Rommelsdorf, West Germany) and centrifuged at 37°C at 300g for 7 minutes. This formed a cell plug containing leucocytes at the bottom of the tube. The supernatant was removed and the remaining red cells were destroyed by hypotonic lysis which was achieved by adding 2ml of water and then 13 seconds later by adding 2ml of a x2 Earle's solution (Gibco, Paisley, UK), thereby suspending the cells in a x1 concentration of Earle's buffer containing (mM): NaCl 116, KCl 5.36, MgSO\(_4\).7H\(_2\)O 0.81 NaH\(_2\)PO\(_4\).H\(_2\)O 1.01, CaCl\(_2\) 1.80, NaHCO\(_3\) 26.19, Fe(NO\(_3\))\(_3\).9H\(_2\)O 0.002.

The percentage of white cell types was calculated by microscopic counting of cells and comprised 70% neutrophils, 18%
lymphocytes, 3% eosinophils and basophils combined, and 9% smear
cells. The cell suspension was centrifuged at 37°C at 300g for 5
minutes and formed a pellet, this was resuspended in 8ml of
tissue culture medium (M199).

A 3ml aliquot of the cell suspension was taken for
subsequent estimation of intracellular electrolytes. The
remaining 5ml was labelled with 5uCi of $^{22}$Na (specific activity
100-1800 Ci/gm, Amersham, Buckinghamshire, UK), and incubated at
37°C for 30 minutes to reach a steady state. At the end of this
time the cells were centrifuged for 3 minutes at 37°C at 300g and
resuspended in 6ml of unlabelled M199 to remove excess
radioisotope from the cells. This procedure was repeated, after
which the cell suspension was split into two aliquots of 3ml
each. To one of these was added 0.1ml of 1mM ouabain (Sigma
Chemicals, Dorset, UK) and samples were taken from both aliquots
at regular intervals over 20 minutes. Each pair of samples was
spun at 2000g to stop sodium efflux and precipitate the cells.
The supernatant was removed, the tube was dried with paper
tissue, and residual radioactivity was counted in a Packard Auto-
Gamma 5650 gamma counter (Canberra Packard, Berkshire, UK). The
total sodium efflux rate constant was calculated from the slope
of the linear regression curve in the absence of ouabain.
Glycoside-sensitive activity (sodium pump activity) was derived
by subtracting the rate constant obtained in the presence of
ouabain from the total.

The 3ml unlabelled aliquot obtained above was allowed to
stand at 37°C for 25 minutes. At the end of this time the cells
were centrifuged at 0°C and 300g for 3 minutes and then
resuspended in 3ml of 99mM ice-cold magnesium chloride ($\text{MgCl}_2$).
The cells were again centrifuged at 0°C and 300g for a further 3 minutes and then resuspended in 1ml of 99mM MgCl₂. The suspension was transferred to a preweighed polythene tube and centrifuged at 2000g for 5 minutes at 0°C. The supernatant was removed and the tube was dried and placed in an oven at 100°C for 12 hours to ash the cells. The tube was reweighed and the ash was placed in deionised water to leach out the sodium and potassium. The sodium and potassium were estimated by flame photometry using a Corning-EEL 450 instrument (Corning, Essex, UK) and the results expressed as mmol per kg of dry weight of cells.

In 12 human subjects the interassay variations were measured on leucocyte sodium movements recorded on two occasions separated by a minimum of 7 days, these were:

- Total efflux rate constant for sodium: 10%
- Ouabain resistant efflux rate for sodium: 28%
- Ouabain sensitive efflux rate for sodium: 14%
- Leucocyte sodium content: 13%
- Leucocyte potassium content: 7%
- Total sodium efflux rate: 17%
- Ouabain resistant efflux rate: 30%
- Ouabain sensitive efflux rate: 24%

**Renin Measurements**

Rat plasma renin concentration was measured according to the method of Thurston et al. (1980). Rat renin substrate was prepared in the following way: bilateral nephrectomy was performed through flank incisions on 250g female Wistar rats under ether anaesthesia. Groups of six to ten rats were prepared at a time. The animals were allowed to recover and left with free
access to water, but not food, for twenty-four hours. After this time they were re-anaesthetised and the lower portion of the aorta was exposed down to the bifurcation through an abdominal incision. As much blood as possible (usually 7 to 10ml) was withdrawn into a syringe containing 500ul of a 10% solution of potassium EDTA. The blood was immediately transferred to containers on ice and then spun at 2,000g for seven minutes at 4°C as soon as possible. The plasma so obtained was pooled and stored at -20°C in 10ml aliquots.

To 10ml of thawed plasma were added 500ul of 1M Tris Maleate (final concentration 50mM) and 500ul of a saturated ethanolic solution of phenylmethyl-sulfonyl fluoride (Sigma Chemicals, Dorset, UK). The pH of the solution was adjusted to 6.5 with 2M NaOH. For plasma renin measurements 100ul of plasma were mixed with 400ul of substrate plasma. The mixture was divided into three aliquots of approximately 150ul. One of these was kept on ice throughout. This was the blank which gave a measure of the angiotensin I already present in the sample before incubation. The remaining two were incubated at 37°C in a shaking water bath for fifteen or thirty minutes. Samples with a high renin concentration were diluted with assay buffer before mixing with substrate plasma. The incubation was stopped by transferring tubes to ice and assayed immediately or stored at -20°C until required.

The angiotensin I generated during the incubation was measured by radioimmunoassay. Lyophilised rabbit angiotensin I antiserum was obtained from Becton Dickinson (Wembley, UK). A dilution of 1:25,000 in assay buffer (100mM Tris Maleate, pH 7.4 containing 0.1 percent radioimmunoassay grade bovine serum
albumen) was made which gave a range of binding between 40 and 70 percent under the assay conditions used. $^{125}$I-labelled angiotensin I (approximately 2000 Ci.mmol$^{-1}$ Amersham International, Buckinghamshire, UK) was also diluted in assay buffer to give 0.2uCi.ml$^{-1}$, equivalent to 10,000cpm.50ul$^{-1}$. The reagents and the reaction mixture were kept cold at all times.

25ul of incubate were diluted with 200ul of assay buffer and mixed with 200ul of antibody and 50ul of $^{125}$I-labelled angiotensin I. Two standard curves were included in each assay consisting of eight tubes containing a range of between 3-125pg and 400pg of angiotensin I (Beckman, Geneva, Switzerland). The standard curves were constructed by serial dilution in assay buffer. Each tube contained 200ul of the appropriate standard. The total activity (To) and the total binding (Bo) were also determined in duplicate for each assay. To is the total of unbound (free) counts measured in the absence of both unlabelled angiotensin I and antibody. Bo is the total of free counts measured in the absence of unlabelled angiotensin I alone. Non-specific binding was less than 5 percent.

The reaction mixture was incubated at 4°C overnight (for a minimum of sixteen or a maximum of twenty-four hours). After this time the remaining free angiotensin I was separated from the reaction mixture by absorption onto dextran coated charcoal. This was made by suspending 1.25g Dextran T70 (Pharmacia Fine Chemicals, Uppsala, Sweden) and 1.50g Charcoal Norit SX1 (Analytical Supplies, Derby, UK) in 500ml distilled water. It was precooled before use and stirred continuously during addition to the incubated samples. 1ml was added to each tube, thoroughly mixed and immediately spun at 2,000g for seven minutes at 4°C.
The supernatant was discarded and the activity in the pellet counted using an automatic gamma counter (Packard A 5650, Canberra Packard, Berkshire, UK). The counting time was adjusted to give 10,000cpm in the To tube. This was usually between fifty-five and seventy-five seconds. All the tubes were then counted for this length of time.

A standard curve was constructed using a logit plot which was used to convert the counts of the meaned duplicates (B) into logit values based on the value of Bo. A straight line plot \( r > 0.990 \) was then obtained of the logit of free counts versus pg of angiotensin I in the standards:

\[
\text{Logit} = \frac{B}{Bo} \frac{1 - B}{1 - Bo}
\]

The counts in the sample tube were then read off this standard curve and converted into pg of angiotensin I. Renin concentrations were expressed as ng angiotensin I.ml plasma\(^{-1}\). hour\(^{-1}\).

**PROTEIN ESTIMATION**

Protein determinations were carried out with slight modifications according to the method of Lowry et al. (1951). 50ul of solubilised protein solution was diluted to 0.5ml in duplicate for assay and a standard curve of protein over the range 12.5ug to 100ug.ml\(^{-1}\).
2.2 ANIMAL EXPERIMENTATION

BLOOD SAMPLING

To obtain a blood sample greater than 2ml rats were given an intraperitoneal injection of sodium pentabarbitone 20mg.kg\(^{-1}\) body weight. When anaesthetised the abdomen was opened, fat was cleared from around the abdominal aorta and a 19 gauge hypodermic needle was inserted into the aorta towards the heart at the bifurcation of the femoral arteries. Blood could then be withdrawn into a 10ml syringe containing an anticoagulant taking care not to collapse the aorta. About 5 to 8mls of blood could be drawn in this way from an adult rat before circulatory failure.

Smaller blood samples of 2ml or less could be taken from conscious rats via indwelling arterial cannulae. This method of sampling allowed the collection of a blood sample free from anaesthetic. Blood was allowed to drain into a plastic tube containing anticoagulant.

BLOOD PRESSURE MEASUREMENT BY TAIL PLETHYSMOGRAPHY

Light plethysmography is an indirect method of blood pressure measurement and has been fully described by Swales and Tange (1970). The method allows the detection of small differences in light translucency in the caudal artery during systole and diastole when a rat's tail is positioned on a photoelectric sensor. The signal from the photoelectric sensor is relayed to an oscilloscope.

Whilst animals were maintained under light ether anaesthesia the rat's tail was passed through a specially designed inflatable cuff, attached to an ordinary mercury sphygmomanometer, and was positioned near the base of the tail. The photoelectric sensor
was also placed on the tail immediately behind the cuff and retained in position by a spring-loaded clamp. The light source was switched on and the pulse appeared on the oscilloscope screen. If there was no pulse, the tail of the animal was rotated slightly until a trace was seen. The sphygmomanometer cuff was gently inflated to occlude the blood flow and the pulse disappeared completely to give a straight line on the oscilloscope screen. The cuff was then deflated slowly until a pulse just reappeared and the blood pressure value was noted from the sphygmomanometer. This technique enabled rapid and reproducible measurements of blood pressure to be made at normal room temperature (20°C-24°C).

MEASUREMENT OF BLOOD PRESSURE BY DIRECT ARTERIAL CANNULATION

Both carotid and femoral artery cannulations were carried out on rats under ether anaesthesia. Carotid artery catheterisation was performed through an incision on the anterior surface of the neck. The carotid catheter (p50, Portex, Kent, UK) had internal and external diameters of 0.58mm and 0.96mm respectively and was inserted then secured in position with three braided silk ties (3/0, Ethicon, Edinburgh, UK). This catheter was exteriorised between the scapulae passing to the right side of the trachea between the muscle layers. For catheterisation of the right femoral artery the procedure was similar; smaller tubing (p25), external diameter 0.8mm, internal diameter 0.4mm, was inserted into the left femoral artery at the body wall, this was made possible by an incision on the inside of the thigh. The polythene tubing was led subcutaneously towards the neck where it was exteriorised between the scapulae. Both femoral and carotid
artery catheters were protected externally by a stainless steel spring, 40cm in length. Four hours after surgery blood pressure recordings were made.

Arterial catheters were connected to a Statham P23 strain gauge transducer (Stagg Instruments, Henley-on-Thames, UK) via a metal tubing adapter (Clay Adams, NJ, USA) and a plastic three-way tap (Vygon, Cirencester, UK). A continuous recording of blood pressure was made using a Grass polygraph recorder (Grass Instruments Co., Quincy, MA, USA) to which the transducer was linked. A continuous recording of both systolic and diastolic blood pressures was thus obtained and mean arterial blood pressure could be calculated as 1/3 pulse pressure. After blood pressure had been recorded for at least 45 minutes the animal was sacrificed.

AORTIC COARCTATION

Coarctation of the aorta was induced under ether anaesthesia via an incision on the right flank using the modified technique of Selye and Stone (1946). A silk ligature was tied around the aorta between the origins of the two renal arteries, with a wire of 0.4mm diameter introduced inside the ligature and withdrawn, thereby allowing a stenosis of constant diameter to be induced. Sham operations were performed by manipulating the aorta between the renal arteries, but no ligature was applied.
Figure 1. The accumulation of inositol phosphate over 120 minutes in presence of 10mM lithium chloride (a) and the effect of changing the dose of lithium chloride on inositol phosphate accumulation (b) in sections of rat aorta where ■ represent mean data points for 3 control incubations and ● represent mean data points for 3 incubations carried out in the presence of 1mM noradrenaline. Standard error bars have been omitted for clarity.
Figure 2. Inositol phosphate accumulation in segments of rat aorta in the presence of increasing doses of noradrenaline (a) and the inhibition of inositol phosphate accumulation in the presence of 1mM noradrenaline and increasing concentration of the α-antagonist prazosin (b).
CHAPTER 3

ERYTHROCYTE MEMBRANE FATTY ACID CONTENT IN ESSENTIAL HYPERTENSIVE PATIENTS, NORMOTENSIVE FIRST-DEGREE OFFSPRING, AND MATCHED CONTROL SUBJECTS
3.1 INTRODUCTION

As the fatty acid content of membrane phospholipids influences the biochemical mechanisms controlling membrane processes including sodium/potassium ATP-ase pump activity, ouabain insensitive sodium fluxes and the calcium/magnesium ATP-ase pump, it was decided to investigate the fatty acid content of plasma membranes from essential hypertensive patients and their normotensive offspring in comparison with matched normotensive control subjects.

3.2 SUBJECTS AND METHODS

Seventeen patients with untreated essential hypertension were studied, 12 of whom were male. All were taking a normal Western omnivore diet and had been thoroughly screened to exclude secondary causes of hypertension. These patients were compared with 16 healthy normotensive controls matched for age, sex and weight (Table 1a), who volunteered to donate blood samples following an article in the local newspaper. Blood pressure was measured in the right arm in the lying and standing positions using a Hawksley Random Zero Sphygmomanometer. The mean of three readings in each position was recorded. In addition to the hypertensive patients experiments were performed on 15 normotensive subjects with one or more first-degree relatives known to be receiving medication for essential hypertension. Of this group 9 were male. These subjects were compared with a separate control group with no family history of hypertension (Table 1b).

Lipid fatty acids were measured as their methyl esters in erythrocytes obtained from a 10ml sample of venous blood as
described in section 2.1 and statistical analysis was performed using Student's unpaired t test. Results were expressed as mean and standard error of the mean (SEM).

3.3 RESULTS

HYPERTENSIVES AND CONTROLS

The two groups of subjects showed no difference in mean age or weight, and had equal sex distribution (Table 1a). All components of blood pressure were significantly higher in the hypertensive group (p<0.001) (Table 1a).

Mean content of the monounsaturated fatty acid oleic acid was significantly higher in the hypertensives compared with controls (18.3 ± 0.3% vs 17.3 ± 0.3%, p<0.05) (Figure 1a). The linoleate level was significantly lower in hypertensive patients compared with controls (11.0 ± 0.4% vs 14.1 ± 0.9%, p<0.01) (Figure 1a). Mean arachidonic acid was raised in hypertensives compared with controls (7.4 ± 0.14% vs 6.9 ± 0.2%, p<0.05). There was no correlation between any fatty acid level and any component of blood pressure (p>0.1).

NORMOTENSIVE RELATIVES AND CONTROLS

These subjects were younger than the group of hypertensives and controls and there was no difference in age, weight or blood pressure between relatives and controls and they had equal sex distribution (Table 1b).

Mean oleic acid content was significantly higher in normotensive subjects with a family history of hypertension compared with controls (20.9 ± 0.2% vs 20.2 ± 0.2%, p<0.05) (Figure 1b). There was no correlation between any fatty acid level
and any component of blood pressure (p > 0.1).

Membrane fatty acid profiles obtained in the hypertensive patients and their control group cannot be directly compared with data shown for the normotensive offspring and controls as the gas liquid chromatograph analytical column and the chromatograph amplifier were replaced during an instrument service.
Table 1. Subject characteristics of hypertensive patients (a), their normotensive offspring (b) and controls (mean values expressed ± SEM).

### (a) Controls Hypertensives

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<tr>
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<td>Males</td>
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<td>Females</td>
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<td>5</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>49 ±4</td>
<td>50 ±3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75 ±4</td>
<td>76 ±4</td>
</tr>
<tr>
<td>BP.(mmHg) Supine Standing</td>
<td>139 ±4 132 ±4</td>
<td>168 ±6 * 166 ±6 *</td>
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<tr>
<td></td>
<td>73 ±3</td>
<td>101 ±2 *</td>
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<tr>
<td></td>
<td>81 ±3</td>
<td>109 ±2 *</td>
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* * p<0.001

### (b) Controls Relatives

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<tr>
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</tr>
<tr>
<td>Age (years)</td>
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<td>25 ±1</td>
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<tr>
<td>Weight (kg)</td>
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<td>68 ±4</td>
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<tr>
<td>BP.(mmHg) Supine Standing</td>
<td>125 ±3 117 ±3</td>
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Figure 1. Erythrocyte membrane fatty acid profiles for hypertensives and controls (a) and for normotensive offspring of hypertensives and controls (b). Data are expressed as means with vertical bars indicating S.E. mean and control groups are represented by single hatching. For abbreviations see Section 2.1.
CHAPTER 4

DIETARY LINOLEIC ACID SUPPLEMENTATION, BLOOD CELL FATTY ACID PROFILES AND SODIUM TRANSPORT
4.1 INTRODUCTION

The exact component responsible for blood pressure reductions in vegetarian diets is unclear, but the increased polyunsaturated fat intake has been implicated. It was decided to examine whether increasing just one dietary constituent such as linoleic acid might effect a change in the intrinsic characteristics of the plasma membrane and thereby influence membrane sodium transport and perhaps also alter blood pressure. Linoleic acid was chosen because this membrane component was reduced in the experiments described in the previous chapter.

4.2 SUBJECTS AND METHODS

PROTOCOL

Twenty-two healthy normotensive volunteers were recruited from hospital staff, university students, and members of the public responding to a local newspaper advertisement. Nine were male, all ate an omnivore diet and none had a family history of hypertension. Table 1 gives the characteristics of the group.

The aim of the study was to determine whether the ingestion of linoleic acid and subsequent incorporation into the cell membrane would change univalent cation handling characteristics. Blood pressure was also monitored as it had been postulated that this is influenced by changes in transmembrane sodium movements. A double blind, placebo controlled crossover design was chosen for the trial. Subjects were randomised to receive active or placebo treatment and crossed over to the second treatment so that any order effect could be assessed. Random numbers were obtained from scientific tables (Documenta Geigy, 1975). On recruitment volunteers were weighed and had their lying and
standing blood pressures recorded. All subjects provided three successive 24 hour urine collections for estimation of sodium and potassium excretion. Linoleic acid was administered as eight safflower seed oil capsules a day. Each capsule contained 500mg oil, of which 72% was linoleic acid. This would be expected to increase the average daily linoleic acid intake by roughly 40%. Placebo capsules were identical with active supplements and contained paraffin. Subjects continued taking capsules for 28 days, and at the end of that time they were reweighed, had their blood pressure measured again and gave venous blood for membrane fatty acid estimation and leucocyte sodium transport studies. A 24 hour urine collection was saved during the last day of the diet for estimation of urinary sodium and potassium excretion. Subjects then had a 28 day washout period before being crossed over to the other capsules, and after 28 days the studies were repeated.

Leucocyte electrolytes and sodium efflux rate constants were measured by the method of Milner et al. (1984) and have been described in section 2.1. Also erythrocyte lipid analysis and urinary sodium and potassium concentrations were performed as previously outlined in section 2.1. Blood pressure was measured with a Hawksley random zero sphygmomanometer, three readings were taken in both the lying and standing positions and the average recorded. Statistical analysis was by non-parametric sign testing on the data obtained at the end of each dietary period, as this study was randomised. Results for blood pressure and sodium transport are presented as means and SEM.
4.3 RESULTS

All subjects completed the study, some felt bloated while taking safflower seed oil capsules but otherwise suffered no ill effects. No subjects noted any change in bowel habit during the placebo period.

WEIGHT AND URINARY ELECTROLYTE EXCRETION

There was no significant change in mean weight while taking placebo compared with safflower oil (67 ± 4 v 67 ± 3 kg). Similarly, neither urinary sodium nor potassium excretion was altered (139 ± 10 v 134 ±13 mmol sodium.24h⁻¹; 71 ± 4.5 v 71 ± 5.0 mmol potassium.24h⁻¹).

FATTY ACID COMPOSITION

In preliminary studies linoleic acid values were measured before and 28 days after the ingestion of the safflower seed oil capsules to make sure that the washout period was long enough. There was no significant difference between the linoleic acid content at baseline and after the washout period (11.73 ± 0.3 v 11.8 ± 0.3%; P=0.76). During the study there was no significant change in the saturated fatty acids palmitic and stearic while taking safflower oil compared with placebo (Table 2). Similarly, values of oleic acid and archidonic acid were unaltered. The content of linoleic acid, however, rose significantly with active treatment (p<0.01) (Table 2).

LEUCOCYTE SODIUM TRANSPORT

During treatment with safflower oil the total leucocyte sodium efflux rate constant showed a small increase owing to a
rise in the ouabain sensitive component, but this did not reach statistical significance (Table 3). Mean intracellular sodium content also increased with safflower oil, but this was not significant. The ouabain sensitive sodium efflux rate, however, showed a significant rise with safflower oil compared with placebo \( (p = 0.39) \) (Table 3).

**BLOOD PRESSURE**

Both systolic and diastolic pressures fell in the supine and standing positions with safflower oil compared with placebo (Table 4). The fall in supine systolic pressure was highly significant \( (p < 0.01) \). There was no correlation between the change in membrane linoleic acid and fall in pressure.

**ORDER EFFECTS**

Data on all variables were analysed for possible treatment order effects after the study was concluded. Comparison of the first and second treatment and placebo periods showed no difference, indicating that there was no order effect.
Table 1. Baseline characteristics of 22 subjects studied (mean value expressed ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>25 ±1</td>
<td></td>
</tr>
<tr>
<td><strong>Weight (Kg)</strong></td>
<td>66 ±3</td>
<td></td>
</tr>
<tr>
<td><strong>BP. (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>128 ±4</td>
<td>120 ±4</td>
</tr>
<tr>
<td>Standing</td>
<td>68 ±3</td>
<td>80 ±2</td>
</tr>
<tr>
<td><strong>Urinary Electrolytes (mmol.24hr⁻¹)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>152 ±8</td>
<td>66 ±3</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Mean ±SEM percentage erythrocyte membrane fatty fatty acid content during treatment with placebo and safflower oil.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Placebo</th>
<th>Safflower oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>25.1 ±0.37</td>
<td>24.9 ±0.32</td>
</tr>
<tr>
<td>18:0</td>
<td>23.1 ±0.18</td>
<td>23.1 ±0.19</td>
</tr>
<tr>
<td>18:1</td>
<td>20.3 ±0.22</td>
<td>20.1 ±0.25</td>
</tr>
<tr>
<td>18:2</td>
<td>16.3 ±0.34</td>
<td>17.0 ±0.28 *</td>
</tr>
<tr>
<td>20:4</td>
<td>14.8 ±0.26</td>
<td>15.1 ±0.21</td>
</tr>
</tbody>
</table>

* p<0.01
Table 3. Mean ±SEM leucocyte sodium efflux rate constant, intracellular sodium, and efflux rate during treatment with placebo and safflower oil.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Safflower oil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intracellular sodium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol.Kg cell dry weight⁻¹)</td>
<td>54 ±3.0</td>
<td>59 ±4.4</td>
</tr>
<tr>
<td><strong>Efflux rate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol.Kg cell dry weight⁻¹.h⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>114 ±8.1</td>
<td>134 ±11.1</td>
</tr>
<tr>
<td>Ouabain insensitive</td>
<td>41 ±6.2</td>
<td>34 ± 5.1</td>
</tr>
<tr>
<td>Ouabain sensitive</td>
<td>72 ±5.1</td>
<td>100 ± 9.3</td>
</tr>
<tr>
<td><strong>Mean efflux rate constant</strong></td>
<td>.h⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.2 ±0.1</td>
<td>2.3 ±0.1</td>
</tr>
<tr>
<td>Ouabain insensitive</td>
<td>0.8 ±0.1</td>
<td>0.6 ±0.1</td>
</tr>
<tr>
<td>Ouabain sensitive</td>
<td>1.4 ±0.1</td>
<td>1.7 ±0.2</td>
</tr>
</tbody>
</table>

* p<0.05
Table 4. Mean ±SEM supine and standing blood pressures (mmHg) during treatment with placebo and safflower oil.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Safflower oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supine</td>
<td>Standing</td>
</tr>
<tr>
<td>Systolic</td>
<td>129 ±3.3</td>
<td>117 ±3.4</td>
</tr>
<tr>
<td>Diastolic</td>
<td>68 ±1.9</td>
<td>75 ±2.1</td>
</tr>
</tbody>
</table>

* p<0.01
CHAPTER 5

INCREASING LINOLEIC ACID SUPPLEMENTATION, MEMBRANE LIPIDS AND
LEUCOCYTE SODIUM TRANSPORT
5.1 **INTRODUCTION**

In view of the finding that a small supplement of dietary linoleic acid could effect changes in membrane sodium transport and blood pressure, it was decided to investigate whether these small changes could be amplified by increasing the dose of polyunsaturated fatty acid administered. Dosage was raised to the upper limit of tolerability.

5.1 **SUBJECTS AND METHODS**

Ten normotensive volunteers were studied, of whom 6 were male and all ate an omnivore diet and were on no drug therapy. The mean age was 25 ± 1.4 years, height 1.72 ± 0.04 m and weight 73 ± 5.2 kg. Mean blood pressure was 124 ± 5/65 ± 4 mmHg supine and 115 ± 4/70 ± 5 mmHg standing. Mean urinary sodium excretion was 157 ± 13 mmol.24 h⁻¹ and potassium excretion was 73 ± 4 mmol.24 h⁻¹.

**STUDY DESIGN AND PROTOCOL**

The study design used was single-blind and non-randomised. Normotensive volunteers were weighed and had their height and blood pressure measured as well as providing three 24 hour urine collections for estimation of sodium and potassium excretion. Anyone not taking a normal omnivore diet was excluded. Subjects were then asked to take 8 placebo capsules per day (containing paraffin) for 14 days, at which point they were reweighed, and blood pressure was measured. A further 24 hour urine save was collected and venous blood was drawn for erythrocyte membrane fatty acid estimation and leucocyte sodium transport studies. Volunteers then took 8 safflower seed oil capsules per day for 14
days at which time the investigations were repeated. Each capsule contained 500mg oil of which 72% was linoleic acid. The number of capsules was then increased to 16 per day for 14 days, then 32 per day and finally 64 capsules daily for the last two weeks. Investigations were repeated every 14 days just before the capsule number was increased.

Extraction, derivitisation and chromatographic analysis of erythrocyte lipid fatty acids was carried out as described in section 2.1. Urinary electrolyte measurements were made using a Corning flame photometer. Blood pressure was measured with a Hawksley random zero sphygmomanometer, three readings were taken in both the lying and standing positions and the average recorded. Results were expressed as mean ± SEM and statistical analysis was performed using analysis of variance.

5.3 RESULTS

All subjects tolerated 32 capsules of safflower seed oil daily, but increasing the dose to 64 per day produced profound nausea in three volunteers who therefore failed to complete the last 14 days of the study.

WEIGHT, BLOOD PRESSURE, URINARY ELECTROLYTE EXCRETION

Mean weight remained stable over the period of the study except in subjects who received the maximum dose, where mean weight was non-significantly increased (Table 1). This apparent increase however was attributable to a lower mean weight in the 3 subjects who dropped out at this stage. Mean blood pressure showed no significant change at any stage (Table 1). Urinary excretion of sodium and potassium was unchanged (Table 1).
**LEUCOCYTE SODIUM TRANSPORT**

Intraleucocytic sodium content was not altered during the study (Table 2). In addition, mean total ouabain sensitive sodium efflux rates fell slightly but did not reach statistical significance (Table 2). However, mean ouabain resistant sodium efflux rate fell significantly as the amount of safflower seed oil increased ($F=3.7, p<0.05$) (Table 2).

**ERYTHROCYTE MEMBRANE LIPIDS**

Red cell membrane palmitic acid, stearic acid and arachidonic acid content was not changed during the study (Fig 1). However, there was a progressive rise in linoleic acid incorporation into the membrane ($p<0.01$, Fig 1) and at the same time oleic acid content fell ($p<0.01$, Fig 1).

Intraleucocytic sodium content was significantly correlated with membrane oleic acid content ($r=0.368, p<0.05$). Similarly total leucocyte sodium efflux rate was correlated with oleic acid ($r=0.416, p<0.01$), as was ouabain resistant sodium efflux ($r=0.453, p<0.01$). Linoleic acid content was negatively correlated with ouabain resistant sodium efflux ($r=-0.319, p<0.005$).
Table 1. Weight, blood pressure and electrolyte characteristics of subjects (mean ±SEM).

<table>
<thead>
<tr>
<th>Capsules (number.day⁻¹)</th>
<th>Placebo</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (Kg)</td>
<td>74 ±5</td>
<td>74 ±5</td>
<td>75 ±5</td>
<td>75 ±5</td>
<td>79 ±7</td>
</tr>
<tr>
<td>BP. (mmHg) supine</td>
<td>129 ±5</td>
<td>130 ±5</td>
<td>128 ±5</td>
<td>132 ±5</td>
<td>135 ±9</td>
</tr>
<tr>
<td></td>
<td>77 ±4</td>
<td>74 ±4</td>
<td>77 ±3</td>
<td>75 ±3</td>
<td>73 ±2</td>
</tr>
<tr>
<td>BP. (mmHg) standing</td>
<td>124 ±5</td>
<td>123 ±5</td>
<td>125 ±5</td>
<td>126 ±6</td>
<td>127 ±9</td>
</tr>
<tr>
<td></td>
<td>84 ±4</td>
<td>82 ±4</td>
<td>86 ±4</td>
<td>83 ±5</td>
<td>85 ±5</td>
</tr>
<tr>
<td>Sodium excretion (mmol.24 h⁻¹)</td>
<td>147±18</td>
<td>175±15</td>
<td>136±18</td>
<td>149±25</td>
<td>145±40</td>
</tr>
<tr>
<td>Potassium excretion (mmol.24 h⁻¹)</td>
<td>79 ±8</td>
<td>74±10</td>
<td>77 ±5</td>
<td>75±10</td>
<td>63±16</td>
</tr>
</tbody>
</table>
Table 2. Subject leucocyte sodium transport parameters. (mean ±SEM).

<table>
<thead>
<tr>
<th>Capsules (number/day)</th>
<th>Placebo</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracellular</td>
<td>40 ±3</td>
<td>39 ±1</td>
<td>36 ±2</td>
<td>37 ±2</td>
<td>35 ±1</td>
</tr>
<tr>
<td>concentration (mmol/Kg cell dry weight⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total efflux rate</td>
<td>105 ±9</td>
<td>90 ±7</td>
<td>91 ±7</td>
<td>83 ±3</td>
<td>83 ±5</td>
</tr>
<tr>
<td>(mmol/Kg dry weight⁻¹.h⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ouabain resistant</td>
<td>40 ±4</td>
<td>30 ±4</td>
<td>27 ±2</td>
<td>30 ±2</td>
<td>24 ±2</td>
</tr>
<tr>
<td>efflux rate (mmol/Kg dry weight⁻¹.h⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Ouabain sensitive</td>
<td>65 ±6</td>
<td>60 ±5</td>
<td>64 ±6</td>
<td>52 ±2</td>
<td>59 ±5</td>
</tr>
<tr>
<td>efflux rate (mmol/Kg dry weight⁻¹.h⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05
Figure 1. Dose dependent changes in erythrocyte membrane fatty acids during dietary supplementation with safflower oil in normotensive volunteers. Data are expressed as means with vertical lines indicating S.E. mean. For abbreviations see Section 2.1.
CHAPTER 6

DIETARY FAT MANIPULATIONS IN RAT GENETIC HYPERTENSION
6.1 **INTRODUCTION**

In this chapter I have investigated erythrocyte and adipocyte fatty acid composition in SH and Wistar-Kyoto (WKY) control rats taking a normal chow diet and two experimental diets containing widely differing linoleic acid contents.

6.2 **ANIMALS AND METHODS**

Female rats of the Okamoto-Aoki strain of SHR were studied, and age-matched female rats of the WKY strain served as controls. At five weeks of age 30 rats of each strain were randomly allocated to one of three equal dietary groups: a high linoleic acid containing diet (HLA), a normal linoleic acid containing diet (NLA) and a control economy rat chow. Animals were housed in nalgene plastic cages with stainless steel screening on the top situated in a room illuminated for 12 hours during the day at a temperature of 23°C. Fresh diet and water were available ad libitum.

The HLA and NLA diets were prepared according to the composition reported by Barcelli et al. (1982) by Labshure Animal Foods (Surrey, UK) and stored at 4°C (Table 1). Linoleic acid constituted 9.1g per Kg of the NLA diet (2% of the caloric content) and 151.9g per Kg of the HLA diet (27% of the caloric content). The total fat carbohydrate and protein content of both these diets was similar (Table 1). The linoleic acid content of the economy chow diet (Labshure) was 3.5g per kg and the total fat content was lower than that of the two experimental diets (Table 1).

Weekly, body weight was recorded and indirect systolic blood pressure was measured under light ether anaesthesia in all
animals in the study. After 13 weeks of dietary treatment polythene catheters were placed in the left carotid artery enabling 4 hours following surgery the subsequent drawing of a 2ml to 3ml blood sample (see section 2.2) into a glass container on ice with lithium heparin as anticoagulant. The animal was then killed by cervical dislocation and, in some animals, a small portion of adipose tissue approximately $2.5\text{mm}^3$ was removed from the lower abdomen around the left kidney for lipid analysis. This tissue was homogenised in 1ml of water ($4^\circ\text{C}$) prior to lipid extraction. Erythrocyte and adipocyte fatty acid measurements were carried out as described in section 2.1. In order to determine whether the age of animals was an important determinant of membrane fatty acid metabolism erythrocyte fatty acids were also measured in SH and WKY rats after a further 8 and 17 weeks feeding on the economy chow diet.

6.3 RESULTS

**DIETS** (Table 1)

HLA and NLA experimental diets were identical in all respects other than the type of fat present. Modifications to the type of fat were made in order to create a different composition of linoleic acid and also to give two different P/S ratios. The P/S ratio for the HLA diet was 3.2, and 0.08 for the NLA diet. The HLA diet contained a higher proportion of polyunsaturated fat and less monounsaturated and saturated fat than the NLA diet. These differences were for the most part confined to fats with carbon chain lengths of 16 and 18. In this way the similarity in amounts of this group of components was preserved, representing 94% of the total fatty acids present in the HLA diet and 89% in
the NLA diet. The economy chow diet differed from the experimental diets in containing one tenth the amount of fat. In addition, the chow diet had a P/S ratio of 0.66 and contained less than half the amount of linoleic found in the NLA diet. (Table 1).

WEIGHT AND BLOOD PRESSURE

Body weight of animals fed the two experimental diets did not differ from that of rats fed the control economy chow diet in either strain, however, WKY rats were significantly heavier on all dietary treatment towards the end of the study (p<0.01) (Figure 1). After 13 weeks of feeding on selected diets, blood pressure was not significantly different in either SH or control rats fed chow or experimental diet. SHR animals taking the economy chow diet characteristically had higher blood pressures than their WKY controls (ANOVA p<0.0001, figure 2). SHRs fed either the HLA or NLA diets had similar blood pressures and these were not significantly different to those of SHRs fed economy chow (Figure 2). Similarly, WKY control rats fed either of the two experimental diets had blood pressures that did not differ from animals fed the economy chow, and there was no difference in blood pressures in either strain between those rats fed HLA and those fed NLA diet (Figure 2). As the development of blood pressure with age did not differ between rats taking the two experimental diets and those rats fed the control economy chow (Figure 2), it appeared that neither the NLA nor the HLA diet at this level of feeding was able to influence blood pressure in these animals.
ERYTHROCYTE FATTY ACIDS

Analysis of erythrocyte lipid fatty acids in SHR and WKY rats following 13 weeks feeding of an economy chow diet revealed no differences in the relative amounts of major fatty acids present (Table 2). In addition, these rats were studied at two further ages; at 26 weeks and at 35 weeks of feeding on an economy chow diet, no difference in the relative amounts of fatty acids was observed between or within the two strains (Table 2). Interestingly, in rats feeding on experimental diet which was of a higher fat content than economy chow, highly significant interstrain differences in erythrocyte fatty acids were found and the majority were similar in rats feeding on HLA and NLA diets (Table 3). SHRs feeding on these diets had larger relative amounts of the saturated fat stearic acid (p<0.01) and smaller amounts of linoleic acid (p<0.01; Table 3). Also, when these rats were fed NLA diet, SHRs had a reduced relative amount of the monounsaturated fat palmitoleic acid (p<0.01; Table 3).

Major differences in erythrocyte fatty acids were found when the HLA diet fed rats were compared with rats fed on NLA, these differences were common to both SHR and WKY rats. Linoleic acid was highly significantly increased by a factor of two in HLA fed rats (p<0.001), which appeared to be mirrored by a reduction in relative amounts of oleic acid (p<0.001; Table 3). Other concomitant differences also appeared to follow a pattern; the saturated fats palmitic and stearic acids were increased (p<0.005) and the monounsaturated fat including oleic acid were decreased (palmitoleic acid, p<0.001). Arachidonic acid was not different in composition in either strain (Table 3).

No correlation between blood pressure and any of the fatty
acids measured was seen in either WKY or SHR rats (p>0.1).

**ADIPOSE FATTY ACIDS**

In a subgroup of both rat species fed the NLA or HLA diets (WKY n = 3, SHR n = 9), adipocyte stored triacylglycerol fatty acid profiles were measured, these showed both expected inter diet differences and unexpectedly highly significant inter strain differences. This tissue possessed a fatty acid profile very different to that found in the erythrocyte. In general there was relatively less polyunsaturated fat and far more saturated and monounsaturated fat.

Compared with animals fed the NLA diet, the adipocytes of HLA diet fed rats contained a relatively larger proportion of polyunsaturated fat (linoleic acid p<0.001; linolenic acid p<0.001; arachidonic acid p<0.01; Table 4), and a smaller proportion of monounsaturated fats (palmitoleic acid p<0.01, oleic acid p<0.001, eicosanoic acid p<0.001; Table 4) and saturated fats (palmitic acid p<0.001, stearic acid p<0.01; Table 4). For the most part these changes were similar to those found in the erythrocyte between the two diets. Inter species differences in adipocyte fatty acid profiles were found in both dietary groups, these appeared to reflect differences seen in erythrocytes; SHRs had an increased proportion of saturated fat (palmitic acid p<0.05, stearic acid p<0.05) and a reduced proportion of linoleic acid (p<0.05) (Table 4). There was no correlation with blood pressure.
Table 1. Diet Constituents (g.kg diet\(^{-1}\)). PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids.

<table>
<thead>
<tr>
<th></th>
<th>CHOW</th>
<th>NLA</th>
<th>HLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat</td>
<td>21</td>
<td>212</td>
<td>212</td>
</tr>
<tr>
<td>Protein</td>
<td>160</td>
<td>190</td>
<td>190</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>580</td>
<td>550</td>
<td>550</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>3.5</td>
<td>9.1</td>
<td>151.9</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>0.66</td>
<td>0.08</td>
<td>3.18</td>
</tr>
<tr>
<td>n(^{-6}) PUFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18</td>
<td>8.3</td>
<td>13.7</td>
<td>161.35</td>
</tr>
<tr>
<td>C20</td>
<td>0</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>n(^{-3}) PUFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;C16</td>
<td>0</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>C16</td>
<td>0</td>
<td>14.7</td>
<td>0.2</td>
</tr>
<tr>
<td>C18</td>
<td>8.2</td>
<td>87.3</td>
<td>27.8</td>
</tr>
<tr>
<td>C20</td>
<td>0</td>
<td>0.02</td>
<td>0.2</td>
</tr>
<tr>
<td>SFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;C16</td>
<td>0</td>
<td>7.7</td>
<td>0</td>
</tr>
<tr>
<td>C16</td>
<td>2.6</td>
<td>56.8</td>
<td>16.7</td>
</tr>
<tr>
<td>C18</td>
<td>1.7</td>
<td>26.7</td>
<td>5.4</td>
</tr>
<tr>
<td>C20</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Table 2. Age and erythrocyte fatty acid profiles for economy chow fed rats (mean ±SEM, 10 animals were present in each group).

<table>
<thead>
<tr>
<th></th>
<th>18 weeks</th>
<th>26 weeks</th>
<th>35 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>49.50 ±0.68</td>
<td>47.85 ±0.88</td>
<td>49.14 ±0.39</td>
</tr>
<tr>
<td>WKY</td>
<td>50.17 ±0.69</td>
<td>48.22 ±0.91</td>
<td>49.66 ±0.38</td>
</tr>
<tr>
<td>SHR</td>
<td>1.09 ±0.12</td>
<td>1.10 ±0.04</td>
<td>1.03 ±0.09</td>
</tr>
<tr>
<td>16:1</td>
<td>0.85 ±0.12</td>
<td>1.00 ±0.14</td>
<td>0.96 ±0.14</td>
</tr>
<tr>
<td>WKY</td>
<td>21.44 ±0.21</td>
<td>22.24 ±0.37</td>
<td>21.64 ±0.17</td>
</tr>
<tr>
<td>SHR</td>
<td>21.82 ±0.16</td>
<td>22.11 ±0.42</td>
<td>21.04 ±0.39</td>
</tr>
<tr>
<td>18:0</td>
<td>9.94 ±0.32</td>
<td>10.21 ±0.22</td>
<td>9.85 ±0.32</td>
</tr>
<tr>
<td>WKY</td>
<td>9.46 ±0.16</td>
<td>10.18 ±0.47</td>
<td>9.95 ±0.33</td>
</tr>
<tr>
<td>SHR</td>
<td>7.10 ±0.10</td>
<td>7.07 ±0.13</td>
<td>7.53 ±0.30</td>
</tr>
<tr>
<td>18:2</td>
<td>6.85 ±0.08</td>
<td>7.23 ±0.36</td>
<td>7.45 ±0.32</td>
</tr>
<tr>
<td>WKY</td>
<td>10.93 ±0.36</td>
<td>11.55 ±0.40</td>
<td>10.91 ±0.26</td>
</tr>
<tr>
<td>SHR</td>
<td>11.02 ±0.54</td>
<td>11.32 ±0.36</td>
<td>10.96 ±0.27</td>
</tr>
</tbody>
</table>
Table 3. WKY and SHR erythrocyte fatty acid profiles during feeding on experimental diets (mean ±SEM).

<table>
<thead>
<tr>
<th></th>
<th>NLA</th>
<th>HLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>38.28 ±0.71</td>
<td>39.72 ±1.31</td>
</tr>
<tr>
<td>16:0 SHR</td>
<td>38.28 ±0.98</td>
<td>39.80 ±1.15</td>
</tr>
<tr>
<td>WKY</td>
<td>1.05 ±0.15</td>
<td>0.56 ±0.19</td>
</tr>
<tr>
<td>16:1 SHR</td>
<td>** 0.84 ±0.12</td>
<td>0.42 ±0.08</td>
</tr>
<tr>
<td>WKY</td>
<td>26.64 ±0.34</td>
<td>27.76 ±0.77</td>
</tr>
<tr>
<td>18:0 SHR</td>
<td>** 27.46 ±0.56</td>
<td>*** 29.17 ±0.50</td>
</tr>
<tr>
<td>WKY</td>
<td>15.42 ±0.59</td>
<td>7.72 ±0.62</td>
</tr>
<tr>
<td>18:1 SHR</td>
<td>15.38 ±0.63</td>
<td>7.78 ±0.29</td>
</tr>
<tr>
<td>WKY</td>
<td>6.03 ±0.47</td>
<td>11.72 ±0.95</td>
</tr>
<tr>
<td>18:2 SHR</td>
<td>** 5.27 ±0.45</td>
<td>** 10.37 ±0.48</td>
</tr>
<tr>
<td>WKY</td>
<td>12.58 ±0.38</td>
<td>12.54 ±0.70</td>
</tr>
<tr>
<td>20:4 SHR</td>
<td>12.76 ±0.36</td>
<td>12.90 ±0.35</td>
</tr>
</tbody>
</table>

** p<0.01  *** p<0.001  * p<0.05  ** p<0.01  *** p<0.001
Between Strain  Between Diet
<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>NLA</th>
<th>HLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>WKY 3</td>
<td>30.33 ±1.49</td>
<td>20.40 ±0.87 ***</td>
</tr>
<tr>
<td></td>
<td>SHR 9</td>
<td>34.06 ±1.13</td>
<td>23.61 ±2.03 ***</td>
</tr>
<tr>
<td>16:1</td>
<td>WKY 3</td>
<td>6.03 ±0.95</td>
<td>2.35 ±0.74  **</td>
</tr>
<tr>
<td></td>
<td>SHR 9</td>
<td>6.19 ±0.69</td>
<td>1.80 ±0.50  ***</td>
</tr>
<tr>
<td>18:0</td>
<td>WKY 3</td>
<td>5.37 ±0.67</td>
<td>2.97 ±0.33  **</td>
</tr>
<tr>
<td></td>
<td>SHR 9</td>
<td>* 6.60 ±0.64</td>
<td>*** 3.94 ±0.32 ***</td>
</tr>
<tr>
<td>18:1</td>
<td>WKY 3</td>
<td>47.13 ±2.62</td>
<td>21.10 ±0.37 ***</td>
</tr>
<tr>
<td></td>
<td>SHR 9</td>
<td>45.35 ±1.29</td>
<td>21.22 ±0.53 ***</td>
</tr>
<tr>
<td>18:2</td>
<td>WKY 3</td>
<td>9.11 ±0.86</td>
<td>52.01 ±1.63 ***</td>
</tr>
<tr>
<td></td>
<td>SHR 9</td>
<td>* 5.89 ±0.92</td>
<td>*** 48.19 ±2.53 ***</td>
</tr>
<tr>
<td>18:3</td>
<td>WKY 3</td>
<td>0.73 ±0.08</td>
<td>0.34 ±0.05  **</td>
</tr>
<tr>
<td></td>
<td>SHR 9</td>
<td>0.73 ±0.05</td>
<td>* 0.27 ±0.04 ***</td>
</tr>
<tr>
<td>20:1</td>
<td>WKY 3</td>
<td>1.14 ±0.08</td>
<td>0.22 ±0.04  ***</td>
</tr>
<tr>
<td></td>
<td>SHR 9</td>
<td>1.06 ±0.12</td>
<td>0.23 ±0.03  ***</td>
</tr>
<tr>
<td>20:4</td>
<td>WKY 3</td>
<td>0.13 ±0.04</td>
<td>0.64 ±0.16  **</td>
</tr>
<tr>
<td></td>
<td>SHR 9</td>
<td>0.11 ±0.02</td>
<td>** 0.49 ±0.06 ***</td>
</tr>
</tbody>
</table>

* p<0.05
** p<0.01
*** p<0.001

Between Strain Between Diet
Figure 1. Body weight profile for rats over the 13 week experimental dietary period.

\[ \triangle \text{SHR chow}, \quad \blacktriangle \text{WKY chow}, \quad \bigcirc \text{WKY HLA}, \quad \bullet \text{WKY NLA}, \quad \square \text{SHR NLA}, \quad \mathbf{\square} \text{SHR HLA} \]

Data points represent means for groups of animals at each time point.

Standard error bars and data for rats taking normal chow during the early phase of the study have been omitted for clarity.

\[ \dagger \quad p < 0.001 \text{ compared with WKY rats.} \]
Figure 2. Mean arterial blood pressure profile for rats over the 13 week experimental dietary period.

\( \triangle \) SHR chow, \( \Delta \) WKY chow, \( \bigcirc \) WKY NLA, \( \bullet \) WKY HLA, \( \square \) SHR HLA, \( \blacksquare \) SHR NLA.

Data points represent means for groups of animals at each time point. Standard error bars and data for rats taking normal chow during the early phase of the study have been omitted for clarity.

\( \dagger \) \( p < 0.001 \) compared with WKY rats.
CHAPTER 7

ERYTHROCYTE MEMBRANE PHOSPHOINOSITIDE METABOLISM

IN HUMAN ESSENTIAL HYPERTENSION
7.1 **INTRODUCTION**

It was decided to investigate $^{32}$P incorporation into the phosphoinositides of patients with hypertension, normotensive offspring of hypertensive patients and matched control subjects, in view of the work above in which I have demonstrated abnormalities of plasma membrane fatty acid composition. Such a disturbance may alter mechanisms which control the activity of the phosphoinositide signalling system.

7.2 **SUBJECTS AND METHODS**

**SUBJECTS**

Eleven patients with essential hypertension were studied, after they had been thoroughly screened for secondary causes of raised blood pressure. Eight of the patients were male and the mean age was 48 ± 4.2 years. The mean height of the group was 1.8 ± 0.01m and the mean weight was 79.4 ± 4.8kg. None of the patients had ever received any antihypertensive medication, and the mean blood pressure was 161 ± 7.9/96 ± 4.5mmHg supine and 167 ± 8.7/107 ± 4.2mmHg standing. These subjects were compared with 11 control subjects with no family history of hypertension, of whom eight were male. The mean age of this group was 49 ± 4.1 years, the mean height 1.7 ± 0.04m and the mean weight 73 ± 5.6kg. The mean blood pressure was 139 ± 3.8/80 ± 3.3mmHg supine and 138 ± 4.9/86 ± 2.0mmHg standing. Blood pressures were recorded using a Hawksley Random Zero sphygmomanometer. Three readings were taken both supine and standing and the mean value was recorded in each position.

In addition to the hypertensive patients, experiments were performed on 15 normotensive subjects with one or more first-
degree relatives known to be receiving medication for essential hypertension. Of this group nine were male, the mean age was $31 \pm 2.8$ years, the mean height $1.7 \pm 0.03m$ and the mean weight $65 \pm 3.9kg$. The mean blood pressure was $126 \pm 4.6/68 \pm 5.8mmHg$ supine and $125 \pm 3.6/74 \pm 3.8mmHg$ standing. The results obtained were compared with those from a separate younger control group with no family history of hypertension in order to minimise the possibility of introducing artefactual differences by using the same mismatched control group. Nine of this group were male, the mean age was $34 \pm 2.8$ years, the mean height $1.7 \pm 0.2m$ and the mean weight $67 \pm 3.4kg$. For this group the mean blood pressure was $124 \pm 4.6/70 \pm 2.9mmHg$ supine and $128 \pm 5.0/72 \pm 3.7mmHg$ standing. It is acknowledged that some misclassification of subjects will have occurred in these groups but this will only serve to reduce the chance of obtaining a significant difference between these two populations when measuring phosphoinositide metabolism.

**METHODS**

On the day of study 10mls of venous blood was taken into a Vacutainer tube containing lithium heparin as anticoagulant (Becton Dickinson, Rutherford, NJ,USA). Erythrocyte membranes were then prepared and the incorporation of $^{32}P$ into polyphosphoinositides was carried out as described in section 2.1. Results are expressed as means and SEM. Statistical analysis was performed using Student's unpaired t-test and the pattern of $^{32}P$ incorporation into Ptd-Ins 4P and Ptd Ins 4,5P$_2$ was compared by two-way analysis of variance.
7.3 RESULTS

ESSENTIAL HYPERTENSIVE PATIENTS

The group of untreated essential hypertensive patients was well matched with a group of middle-aged control subjects. There was no significant difference in $^{32}P$ incorporation into Ptd-Ins $4P$ (hypertensive patients vs controls: $0.12 \pm 0.03$ vs $0.16 \pm 0.03$ nmol of $^{32}P$ $\text{mg}^{-1}$ of protein in 15 min) or into Ptd-Ins $4,5P_2$ ($0.21 \pm 0.03$ vs $0.26 \pm 0.03$ nmol of $^{32}P$ $\text{mg}^{-1}$ of protein in 15 min). Analysis of variance failed to show any difference in the pattern of $^{32}P$ incorporation between the two groups.

NORMOTENSIVE OFFSPRING OF HYPERTENSIVE PATIENTS

Incorporation of $^{32}P$ into erythrocyte Ptd-Ins $4P$ was higher in the offspring compared with control subjects but this did not attain statistical significance ($0.20 \pm 0.05$ vs $0.11 \pm 0.01$ nmol of $^{32}P$ $\text{mg}^{-1}$ of protein in 15 min). However, incorporation of $^{32}P$ into erythrocyte Ptd-Ins $4,5P_2$ was significantly higher in offspring ($0.39 \pm 0.08$ vs $0.18 \pm 0.02$ nmol of $^{32}P$ $\text{mg}^{-1}$ of protein in 15 min, $p<0.05$). Two-way analysis of variance demonstrated a highly significant increased rate of $^{32}P$ incorporation in cells from relatives compared with control subjects ($p<0.01$).

There was no significant correlation between the age of control subjects and the rate of $^{32}P$ incorporation into Ptd-Ins $4P$ ($r=0.26$) or into Ptd-Ins $4,5P_2$ ($r=0.33$) ($n=25$). Within the group of hypertensive subjects age was not associated with the rate of $^{32}P$ incorporation into phosphoinositides (Ptd-Ins $4P$, $r=0.46$; Ptd-Ins $4,5P_2$, $r=0.06$, $n=11$). Similarly, there was no relationship with age and $^{32}P$ incorporation in the normotensive offspring of hypertensive patients (Ptd-Ins $4P$, $r=-0.152$; Ptd-Ins $4,5P_2$, $r=0.16$, $n=15$).
CHAPTER 8

PHOSPHOINOSITIDE METABOLISM IN RAT AORTA
8.1 **INTRODUCTION**

Thus far, my experiments have been confined to circulating blood cells and adipose tissue, neither of which is considered to be intimately involved in the day to day processes that control blood pressure. Therefore, it was important to examine cellular process in vascular tissue. Because of the increasing evidence that activation of inositol phospholipid metabolism may be involved in signal transduction mediated by $\alpha$-receptor activation it was decided to investigate this phenomenon in vascular tissue from SHR and WKY rats, where differences in cell plasma membrane structure have been demonstrated, in order to examine whether this pathway is abnormal in animals genetically at risk of hypertension.

8.2 **METHODS**

Male SHRs aged 5 and 19 weeks were used and compared with matched WKY control animals. Blood pressures were measured by tail plethysmography.

The method used to study phosphoinositide metabolism in the aorta was modified from that described by Berridge et al. (1982) and involves the measurement of accumulated inositol phosphate in the presence of lithium ions. On the day of study animals were killed by cervical dislocation, the thoracic aorta dissected free and the assay was carried out as described in section 2.1. In this manner in 19 week animals a dose-response curve could be constructed from each thoracic aorta obtained. However, 5 week old rats were too small to allow a full dose range to be tested. Therefore it was decided to study phosphoinositide hydrolysis in these animals in the absence and in the presence of noradrenaline.
at 100μM only. All experiments in SHRs were performed at the same time as a control animal was studied. Results are expressed as mean ± SEM and agonist-induced responses expressed as percentage of basal cpm per mg (wet weight) of aorta. Results between SHR and WKY control groups were compared by means of Student's unpaired t test in 5 and 19 week animals. In addition in 19 week old animals the dose response curves were compared by means of two way analysis of variance.

8.3 RESULTS

The mean blood pressure was significantly higher in SHR (n=10) compared with WKY (n=10) at 5 and 19 weeks (5 weeks: 123 ± 4 vs 110 ± 3 mmHg, p<0.03; 19 weeks: 167 ± 4 vs 121 ± 5 mmHg, p<0.001). However, the weights did not differ significantly between the two groups at either time (SHR vs WKY: 5 weeks: 99 ± 5 vs 90 ± 2 g; 19 weeks: 357 ± 6 vs 352 ± 6 g).

[^H]-INOSITOL METABOLISM

Five week old rats

Unstimulated [^H]-inositol phosphate accumulation was significantly higher in SHR compared with WKY control animals (p<0.05, Table 1). Incubation with noradrenaline (0.1mM) resulted in increased inositol phosphate accumulation in both groups and this was significantly higher in SHR (p<0.05, Table 1).

Nineteen week old animals

Basal [^H]-inositol phosphate accumulation was now no longer significantly different in the two groups (Table 1), but there
was a dose-dependent stimulation of $[^3\text{H}]$-inositol phosphate accumulation with noradrenaline in both groups of animals and this was significantly reduced in SHR compared with WKY ($p<0.05$, Table 1, Figure 1), although the $ED_{50}$ was not different in the two strains (SHR vs WKY: $2.02 \pm 0.5$ vs $3.0 \pm 1.1\text{uM}$, n=10 in each group).
Table 1. $[^3\text{H}]$-inositol phosphate production in 5 and 19 week old WKY and SHR rats in the absence and presence of noradrenaline (mean ±SEM, n=10).

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Noradrenaline (0.1mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>[(^3\text{H})-inositol phosphate accumulation](c.p.m./mg tissue wet weight(^{-1}))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5 weeks</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>430 ±47</td>
<td>1695 ±329</td>
</tr>
<tr>
<td>SHR</td>
<td>656 ±92 *</td>
<td>2172 ± 92 *</td>
</tr>
<tr>
<td><strong>19 weeks</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>798 ±130</td>
<td>3726 ±542</td>
</tr>
<tr>
<td>SHR</td>
<td>887 ± 98</td>
<td>2399 ±129 *</td>
</tr>
</tbody>
</table>

*p < 0.05
Figure 1. Noradrenaline dose dependent accumulation of inositol phosphate in aortic sections taken from 19 week old WKY (○) and SHR (□) rats.
Data are expressed as means with vertical bars indicating S.E. mean.
CHAPTER 9

COARCTATION HYPERTENSION AND PHOSPHOINOSITIDE HYDROLYSIS

IN THE RAT
9.1 INTRODUCTION

One difficulty in studying a proposed mechanism which may generate increased blood pressure in genetic hypertension is in dissociating the effect of the mechanism on blood pressure from the effect upon the mechanism of increased blood pressure. One way by which this may partially overcome is to induce hypertension in a normotensive rat, in such a model the effect of pressure upon phosphoinositide metabolism may be studied.

Chronic hypertension is associated with an increase in the dimensions of the media of both small and large arteries (Folkow, 1978). Although these vascular changes appear in some degree in response to pressures (Folkow, 1982; Mulvany, 1987), it has been argued that the smooth muscle cells of genetically hypertension prone rats and humans with a familial predisposition to hypertension display abnormal mitotic features when exposed to pressor or trophic stimuli (Folkow, 1986). The cellular processes responsible for the trophic response remain to be fully characterised. In this regard there has been considerable interest in the plasma membrane polyphosphoinositide signalling system which appears to play an important role in one of the two major pathways involved in cell growth (Berridge, 1987). Of possible relevance to the pathogenesis of hypertension is the fact that processes mediating vasoconstriction may also be mediators of trophic change.

Coarctation of the rat aorta is known to increase blood pressure proximal to the stenosis; if a clip is placed between the kidneys, the distal kidney becomes ischaemic and renin release rises generating high circulating levels of angiotensin II (Carretero et al. 1971). Therefore, this model allows a close
examination of two influences upon vascular growth, i.e. increased load upon the proximal aorta and raised blood angiotensin II concentrations. Accordingly, it was decided to investigate the effects of acute coarctation hypertension upon aortic phosphoinositide hydrolysis and structure in Wistar rats.

9.2 METHODS

ANIMALS

Female Wistar rats (180-210gm body weight) maintained upon normal rat chow were used throughout the study. Coarctation of the aorta and sham operations were carried out as described in section 2.2. All experiments were performed at 3, 9 and 20 days after the surgical procedure. Animals were weighed, blood pressures were measured by direct cannulation of left carotid and right femoral arteries under ether anaesthesia (see section 2.2). Recording of pressures was made 4 hours after surgery to allow the animals to regain consciousness. Approximately 1ml of arterial blood was drawn from conscious rats into a plastic tube on ice containing 100ul of K₂EDTA through a polythene cannula placed in the right femoral artery. Plasma was fractionated by centrifugation at 4°C (15 minutes 2000g) and stored at -20°C prior to estimation of plasma renin concentration using the method of Thurston et al. (1980) (see section 2.1).

PHOSPHOINOSITIDE HYDROLYSIS

On the day of study animals were killed by cervical dislocation and a 4mm segment of aorta taken from above and below the coarctation for measurement of inositol phosphate accumulation in the presence of lithium ions and in the absence
of agonist as described in section 2.1.

MORPHOLOGY OF RAT AORTA

Animals were anaesthetised with an intra abdominal injection of sodium pentabarbitone 20mg.kg$^{-1}$ body weight and were infused through an indwelling carotid cannula with 15mls of a fixative solution over a two minute period which consisted of a physiological salt solution containing 1% formaldehyde 2% glutaraldehyde and (mM): NaCl 119, KCl 4.7, CaCl$_2$.2H$_2$O 2.5, MgSO$_4$.7H$_2$O 1.17, NaHCO$_3$ 25, KH$_2$PO$_4$ 1.18, EDTA 0.026, C$_6$H$_{12}$O$_6$ 5.5.

Two millimetre lengths of aorta were taken and fixed in 2% glutaraldehyde and embedded in epoxy resin. Evaluations of aortic medial thickness and cross-sectional area were carried out on lum sections stained with toluidene blue. Medial thickness was defined as the distance between the mid-points of the internal and external elastic laminae and was measured at 90° intervals around the circumference of each vessel (10 sections for each animal). Medial cross-sectional area was determined by planimetry using a Kontron Videoplan system (Kontron Instruments, Everett, MA, USA). Nuclear profiles per 10,000 u$^2$ were estimated on the same sections used for measuring medial thickness and cross-sectional area using the method of Owens and Schwartz (1982).

Measurements of inositol phosphate production were performed on groups of 12 animals at each time point. Not all measurements could be made on each animal. Therefore, group sizes varied and are given in the tables which describe the results.

Results are expressed as mean ± SEM. Data between groups were analysed using Student's unpaired t test for individual time points and by analysis of variance to include all time points overall.
RESULTS

Following surgery, animals with coarctation lost weight and were significantly lighter than sham operated rats at 3 days (Table 1). Over the next 17 days the experimental group gained weight but were always significantly lighter than the sham control animals (Table 1). Plasma renin concentration was significantly raised at 3, 9 and 20 days (Table 1) compared with controls.

Carotid mean arterial pressure was significantly higher in rats with coarctation at 3 days compared with control rats ($p<0.05$, Figure 1). Thereafter blood pressure rose further in experimental animals, and the overall blood pressure response was highly significantly different by analysis of variance ($p<0.001$). Conversely at 3 days femoral mean arterial pressure fell in rats after coarctation and remained lower at 9 and 20 days (Figure 1). Analysis of variance demonstrated a significant difference in femoral blood pressure between experimental and control rats (Figure 1) ($p<0.001$).

MORPHOLOGY OF THE AORTA

MEDIAL THICKNESS

Above the coarctation aortic medial thickness was unchanged at 3 days compared with sham operated rats (Figure 2). However, at 9 days in ligatured rats there was a significant increase in media thickness which was maintained at 20 days (Figure 2). Below the coarctation medial thickness was unchanged at 3 and 9 days, but was reduced significantly at 20 days (Figure 2).
AORTIC CROSS SECTIONAL AREA

At 3, 9 and 20 days rats with coarctation showed a significant increase in aortic cross sectional area above the ligature compared with control animals (Table 2). Below the ligature, there was no difference in cross sectional area at 3 and 20 days but the cross sectional area was significantly increased at 9 days (Table 3).

AORTIC NUCLEAR COUNTS

These were performed upon segments of aorta proximal to the ligature. At 3 days nuclear counts were not different between experimental and sham operated rats (Table 2). However at 9 and 20 days nuclear counts were significantly lower in rats with coarctation (Table 2) (p<0.001).

AORTIC INOSITOL PHOSPHATE ACCUMULATION

Proximal to the coarctation, aortic inositol phosphate accumulation was raised at 3 days but not significantly different compared with sham operated rats (p = 0.099) (Figure 3a). However, at 9 and 20 days this was significantly increased in rats with coarctation (p<0.01) at both times (Figure 3a). Below the coarctation inositol phosphate accumulation was not different at any time compared with control rats (Figure 3b).
Table 1. Mean ± SEM body weight and plasma renin concentration of rats with coarctation and sham-operated control animals (7 animals were present in each group).

<table>
<thead>
<tr>
<th>TIME (days)</th>
<th>BODY WEIGHT (grams)</th>
<th>PLASMA RENIN CONCENTRATION (ng AII.ml⁻¹.hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Coarc.</td>
</tr>
<tr>
<td>3</td>
<td>192 ±2</td>
<td>*** 165 ±4</td>
</tr>
<tr>
<td>9</td>
<td>205 ±3</td>
<td>*** 175 ±5</td>
</tr>
<tr>
<td>20</td>
<td>242 ±7</td>
<td>** 213 ±6</td>
</tr>
</tbody>
</table>

** p<0.01
*** p<0.001
Table 2. Mean ±SEM aortic cross-sectional area (CSA) and nuclear counts proximal to the ligature site in rats with coarctation and in sham-operated control animals (7 animals were present in each group).

<table>
<thead>
<tr>
<th>TIME (days)</th>
<th>AORTIC CSA (um³)</th>
<th>AORTIC NUCLEAR COUNTS (counts /10,000 um²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHAM</td>
<td>COARC.</td>
</tr>
<tr>
<td>3</td>
<td>0.37±0.003</td>
<td>0.45±0.020 *</td>
</tr>
<tr>
<td>9</td>
<td>0.38±0.006</td>
<td>0.54±0.039 **</td>
</tr>
<tr>
<td>20</td>
<td>0.38±0.007</td>
<td>0.54±0.042 **</td>
</tr>
</tbody>
</table>

* p<0.05  
** p<0.01  
*** p<0.001

Table 3. Mean ±SEM aortic cross-sectional area (CSA) distal to the ligature site in rats with coarctation and in sham-operated control animals (7 animals were present in each group).

<table>
<thead>
<tr>
<th>TIME (days)</th>
<th>AORTIC CSA (um³)</th>
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<tr>
<td></td>
<td>SHAM</td>
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<tr>
<td>3</td>
<td>0.17 ±0.006</td>
</tr>
<tr>
<td>9</td>
<td>0.18 ±0.004</td>
</tr>
<tr>
<td>20</td>
<td>0.22 ±0.017</td>
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** p<0.01
Figure 1. Mean arterial blood pressure in coarctated (open symbols) and sham-operated (closed symbols) animals measured in the carotid (circles) and femoral (squares) arteries at 3, 9 and 20 days following surgery. Data are expressed as means with vertical bars indicating SE mean (n = 7 for each group).
Figure 2. Medial thickness in sections of aorta taken proximal (light hatching) and distal (dark hatching) to the ligature site in coarctated (☐) and sham-operated control rats (☒) at 3, 9 and 20 days following surgery. Data are expressed as means with vertical bars indicating SE mean (n = 7 for each group).
Figure 3. Inositol phosphate accumulation in sections of aorta taken proximal (a) and distal (b) to the ligature site in coarctated (▲) and sham operated control rats (□) at 3, 9 and 20 days following surgery. Data are expressed as means with vertical bars indicating S.E. mean (n = 12 for each group).
CHAPTER 10

ANALYSIS OF PHOSPHOINOSITIDE AND INOSITOL PHOSPHATES

BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
10.1 **INTRODUCTION**

There have now been a number of investigations of phosphoinositide metabolism in arteries (Campbell et al. 1985; Rapoport, 1986) as well as in cells cultured from such tissue (Griendling et al. 1986). The gross accumulation of inositol phosphates observed when experiments are conducted in the presence of lithium ions as documented in preceding chapters, provides a sensitive assay of inositol phospholipid hydrolysis (Berridge et al. 1982) and facilitates the characterisation of pharmacologically active agents which may stimulate or have inhibitory roles upon the hydrolysis of phosphoinositide and vascular contraction (Rapoport, 1986; Fox and Friedman, 1987). In studies of cardiovascular diseases it is necessary to examine this system in contractile vascular tissue rather than in conduit vessels where the majority of resistance to blood flow is located. Conventional low pressure ion exchange techniques used to separate phosphorylated components of this signalling system are not adequate to resolve structural isomers of inositol phosphates. Therefore, in order to describe qualitative and quantitative agonist evoked changes in phosphoinositide signalling in resistance arterioles high performance ion exchange chromatography was applied to try and facilitate examination of inositol containing components of this system in resistance arterioles.

10.2 **METHODS**

Adult Wistar rats (body weight 250-300g) were used for all the experiments described. Resistance vessels were taken, dissected and incubated as described in section 2.1.
10.3 RESULTS

AQUEOUS TISSUE EXTRACTS

Chromatographic analysis of aqueous tissue extracts revealed the presence of six radioactive inositol containing peaks. These were identified by way of their relative retention times to cytidine, adenosine and guanosine phosphate markers as glycerophosphoinositol (GroP-Ins), inositol 1-phosphate (Ins 1-P), inositol 1,4-bisphosphate (Ins 1,4-P$_2$), inositol 1,3,4-trisphosphate (Ins 1,3,4-P$_3$), inositol 1,4,5-trisphosphate (Ins 1,4,5-P$_3$) and inositol 1,3,4,5-tetrakisphosphate (Ins 1,3,4,5-P$_4$) (Figure 1). Authentic $[^3]$H-Ins 1,4,5-P$_3$ was used to confirm the elution position of this compound. In the light of recent reports it is almost certain that each peak of radioactivity in my chromatographic analysis would contain more than one inositol phosphate isomer coeluting with those phosphates indicated (Michell 1986).

Following stimulation with 1mM noradrenaline for 20 seconds, radioactivity associated with aqueous inositol containing compounds changed dramatically. An increase in the radioactivity associated with all five inositol phosphates was noted and this was highly significant for Ins 1,4-P$_2$ ($p<0.001$), and Ins 1,3,4, P$_3$ ($p<0.001$) but not so for Ins 1-P ($p<0.02$), Ins 1,4,5-P$_3$ (NS) and Ins 1,3,4,5-P$_4$ (NS) (Figure 2).

TISSUE DEACYLATED LIPID EXTRACTS

Following chromatographic separation of deacylated tissue extracts, four inositol containing peaks were detected; two of these eluted with the predicted retention times of GroP-Ins and glycerophosphoinositol 4-phosphate (GroP-Ins 4-P). Two peaks were
located in the region of the eluting gradient associated with the expected retention time of glycerophosphoinositol 4,5-bisphosphate (GroP-Ins 4,5-P₂) (Figure 3).

The possible existence of two structural isomers of Ptd-Ins P₂ has recently been investigated in rat parotid glands by Hawkins et al. (1986) and Downes et al. (1986). These authors identified only three inositol containing compounds present in lipid extracts and these were identified as Ptd-Ins, Ptd-Ins 4-P and Ptd-Ins 4,5-P₂. Using an identical lipid deacylation procedure I revealed the existence of a possible Ptd-Ins P₂ structural isomer present in rat resistance arterioles (Figure 3). In order to further identify peaks associated with Ptd-Ins P₂ glycerol ester, a single lipid extract was treated with sodium periodate and 1,1-dimethylhydrazine to remove the glycerol moieties (as described in chapter 2.1), identification of the resulting inositol head group should then be possible on subsequent chromatographic analysis. Following this treatment three of the four inositol containing peaks, on chromatography, co-eluted with Ins 1-P, Ins 1,4-P₂ and Ins 1,4,5-P₃ (Figure 4). However, the peak which had eluted in fraction 58 remained intact following treatment suggesting that this compound probably was not a glycerol ester (Figure 4), and so did not appear to represent the postulated deacylated isomer of Ptd-Ins-P₂. Identification of the elution position of GroP-Ins 4,5-P₂ was then possible and this was found to be associated with the peak eluting in fraction 65 (Figures 3 and 4).

Following exposure to noradrenaline radioactivity associated with all four inositol containing lipid extract components was reduced, and this was most evident for the polyphosphoinosotide
glycerol esters GroP-Ins 4-P (p<0.05) and GroP-Ins 4,5-P2 (p<
0.02) (Figure 5). Radioactivity in the unknown peak eluted in
fraction 58 was also reduced by 40% following agonist exposure
although this did not attain statistical significance (Figure 5).
The net loss of 768 fmol.mg tissue protein⁻¹ of labelled Ptd-Ins
4-P, Ptd-Ins 4,5-P2 and an unknown inositol containing
hydrophobic compound on stimulation was associated with the net
gain of 773 fmol.mg tissue protein⁻¹ of labelled inositol bis-, tris-, and tetrakis- phosphates. By far the greatest increase in
inositol phosphate on stimulation of resistance arterioles was
found in the Ins 1,4-P2 peak. However, reductions in the amount
of labelled polyphosphoinositide were similar for both Ptd-Ins 4-
P and Ptd Ins 4,5-P2. The reduction in radioactivity found in the
peak corresponding to Ptd-Ins was not significant, it was
however, greater than the increase in tissue Ins 1-P.
Figure 1. H.p.l.c. chromatogram showing the resolution of 6 inositol containing peaks in a resistance arteriole aqueous extract following pre-labelling with [3H]-inositol. Beyond fraction 60 the peaks were smaller in comparison with those eluted earlier. Therefore the scale has been altered (0-400cpm) for clarity and included in the figure. For abbreviations in this and subsequent figures see chapter 1.
Figure 2. The changes in the liberation of inositol phosphates following exposure of labelled resistance arterioles to vehicle (single hatching) or 1mM noradrenaline (double hatching) for 20 seconds. Data are expressed as means with vertical bars indicating SE mean (n = 5 for each group).
Figure 3. H.p.l.c. chromatogram showing the resolution of inositol containing components in a resistance arterigle deacylated lipid extract following pre-labelling with $^{3}H$-inositol.
Figure 4. H.p.l.c. chromatogram showing a resistance arteriole deacylated lipid extract following removal of glycerol moieties. At fraction 29 the scale has been altered (0-1x10^{-3}[^3]H-cpm) for clarity. Esterified glycerol was removed by sodium periodate and 1,1-dimethylhydrazine treatment, the sample was then diluted four fold prior to chromatography.
Figure 5. Noradrenaline-induced changes in resistance arteriole inositol containing lipid extract components following deacylation. Data are expressed as means for control (single hatching) and agonist-stimulated (double hatching) incubations. Vertical bars indicate SE means (n = 5 for each group).
CHAPTER 11

DISCUSSION OF RESULTS
These results demonstrate differences in the membrane fatty acid content of red cells between hypertensive patients and controls, and between normotensive offspring of hypertensives and their controls. All subjects were taking an omnivore diet.

Oleic acid was increased both in hypertensive patients and their normotensive first-degree offspring compared with their respective control groups; this was intriguing because the erythrocyte is not intimately involved with blood pressure control and the presence of this abnormality in normotensive subjects genetically prone to the development of hypertension suggests that a genetically determined phenomenon was being observed. A decrease in the level of linoleic acid and an increase in arachidonic acid was found also in hypertensive subjects. Wood et al. (1984) noted a reduced linoleic acid content in adipocytes with increased oleic acid in platelets from patients with coronary heart disease. Indeed more recent work from the same group has demonstrated progressive inverse relations between adipose linoleic acid and the estimated risk of angina pectoris (Wood et al. 1987). In addition, adipocyte linoleic acid was weakly and inversely associated with systolic and diastolic blood pressure, although no such correlations were found in my study.

An inherited defect in membrane handling of fatty acids could have profound effects on plasma membrane structure and function. Phospholipids such as the highly metabolically active phosphoinositides are dependent upon the membrane composition of fatty acids, and a change in the unsaturated fat component can
affect their function (McPhail et al. 1984). Moreover deranged phosphoinositide activity has been reported in hypertension (Marche et al. 1985). Such phospholipids not only control sodium and calcium pump activity, but also determine membrane fluidity and calcium binding to the inside of the plasma membrane, both of which are abnormal in patients with hypertension (Swales, 1982). The significance of the decreased polyunsaturated fat linoleic acid, the raised monounsaturated fat oleic acid and the polyunsaturated arachidonic acid is uncertain. There are two possibilities: there may be a defect in incorporation of fatty acids into the plasma membrane, or alternatively, decreased linoleate may reflect quantitative changes in membrane lipid metabolism including an increased rate of conversion to arachidonic acid and this may lead to an increase in prostaglandin production. However, the opposite appears to be the case (Lukacsko et al. 1980). The possibility that these abnormalities in membrane lipid composition represent a genetically mediated abnormality which may explain both disturbances of ion fluxes and vascular reactivity abnormalities is intriguing and readily lends itself to further testing by appropriate dietary manipulations which were carried out and are discussed below.

**DIETARY LINOLEIC ACID SUPPLEMENTATION, BLOOD CELL FATTY ACID PROFILE AND SODIUM TRANSPORT**

This study shows that when the normal omnivorous diet of healthy volunteers was supplemented with linoleic acid, the cellular membrane handling of sodium could be changed. In addition, a small fall in blood pressure occurred, and both variables were influenced while salt intake and body weight
remained constant. The dietetic manipulation induced a change in absolute sodium efflux and not rate constant indicating no effect upon the Na⁺/K⁺ ATP-ase pump. Therefore, it is assumed that the mechanism by which the change in efflux was effected was by an alteration in sodium influx to which the sodium pump was normally responding. Furthermore, it is presumed that the mechanism by which the influx of sodium was increased would be the incorporation of linoleic acid into the plasma membrane. Indeed, the index of an incorporation of fat into the plasma membrane was a rise in erythrocyte membrane linoleic acid; this tissue was used because the cells are relatively free of organelles whose membrane lipid composition might differ from that of the plasma membrane itself. It is unlikely that the long plasma half life of these cells complicated these studies, as the fat composition of cell membranes varies with dietary intake (Kernoff et al. 1977). The washout period was also shown to be enough for the membranes to adjust from the dietary change. Sodium transport studies were performed upon the leucocyte, a more metabolically active cell but the one which has been shown to have depressed glycoside sensitive sodium efflux in patients with essential hypertension. It is assumed that the changes in lipid profile seen in the erythrocyte reflect the situation in the leucocyte. In terms of sodium transport this would appear to be acceptable (El Ashrey et al. 1989).

The observed fall in blood pressure were small, and only statistically significant with respect to the supine systolic component. The power of the study was such that there was an 80% chance of detecting a fall of 6mmHg mean lying and 9mmHg standing diastolic pressure. Therefore, ameliorative effects on other
components of blood pressure cannot be excluded. However, the failure to reproduce them in the subsequent experiment implies that their significance is uncertain.

With regard to the significance of lipid structure and function in blood pressure homeostasis, the small falls in blood pressure observed in this study do not negate the possible importance of phospholipid metabolism. Whilst the configuration of the lipids may be of importance in this context their influence may be much more specific. Thus, overall changes in P/S ratio have been demonstrated to be effective in lowering blood pressure, but to implicate lipid changes as important may be premature: energy intake and fruit and vegetable consumption are also changed in such dietetic interventions (Beilin, 1987).

In addition, Margetts and co-workers were unable to influence blood pressure in a group of volunteers by increasing the polyunsaturated to saturated fat ratio without altering overall fat intake (Margetts et al. 1985). Thus the nature of the dietary influence on blood pressure is thus uncertain. In this experiment one dietary variable was changed and resulted in a reduction in blood pressure by a comparable extent to that produced by adopting a vegetarian diet (Rouse et al. 1983). Similarly, using n-6 fatty acids may not have been the most appropriate nutrient change to effect. It is interesting that supplementation of a normal Western diet with fish oils, which is rich in long chain n-3 polyunsaturate fatty acids, also produces more significant falls in pressure (Lorenz et al. 1983; Norris et al. 1986). The former study also failed to change erythrocyte sodium co-transport or countertransport characteristics (Lorenz et al. 1983). The properties of complex phospholipids are
dependent on the fatty acid make up of the membrane, (Roelofsen, 1981) and both dietary supplements may well have altered their metabolism. Recent evidence suggests that these play an important part in the regulation of intracellular calcium (Tokumura et al. 1985) and so may be important in the regulation of vascular contractility.

**INCREASING LINOLEIC ACID SUPPLEMENTATION, MEMBRANE LIPIDS AND LEUCOCYTE SODIUM TRANSPORT**

These data demonstrated that increasing the supplementation of a normal omnivore diet with safflower seed oil led to a rise in linoleic acid and a fall in oleic acid content in the erythrocyte plasma membrane in a dose-dependent manner. However, contrary to expectation, total leucocyte sodium efflux was unchanged, as was leucocyte sodium content. Ouabain resistant sodium efflux fell significantly as the linoleic acid supplementation was increased, and there was a significant negative correlation between membrane linoleic acid and ouabain resistant sodium efflux. Mean body weight and urinary sodium and potassium excretion did not change throughout the study suggesting stable sodium and water balance despite the increase in dietary linoleic acid.

The reduction of sodium extrusion found in patients with essential hypertension has been attributed to a humoral inhibitor of sodium/potassium-ATPase. However, the exact identity of such an inhibitor has proved elusive. Indeed, many circulating substances modulate the sodium pump and a number serve as inhibitors (Haber and Haupert, 1987). Recently there have been reports suggesting that fatty acids also might fulfill this role. In particular, unsaturated fats such as linoleic, arachidonic,
linolenic and docosahexaenoic acids have all been implicated in sodium/potassium-ATPase regulation in the electric organ of electrophorus electricus (Bidard et al. 1984), and linoleic and oleic acids in inhibition of sodium transport by acute volume expansion of hog plasma (Tamura et al. 1985). It has been argued that these fatty acids exert an inhibitory effect only in concentrations above the physiological range (Haber and Haupert, 1987). However, Swann has suggested that free fatty acids could be mobilised in excess by phospholipases during hypoxia for example, thereby providing sufficient concentrations to inhibit sodium pump activity (Swann, 1984). This study provides no evidence for linoleic acid as a natriuretic hormone (Tamura et al. 1985). It is of interest that Ng and Hockaday demonstrated reduced ouabain sensitive sodium efflux rate constant in leucocytes exposed to 100uM linoleic acid (Ng and Hockaday, 1986). Studies in which cells were incubated with synthetic medium rather than subjects' own plasma are not directly compatible. However, it is clear that increasing the membrane content of this fatty acid failed to influence sodium extrusion by the sodium/potassium-ATPase pump.

The decrease in ouabain resistant sodium efflux found in these subjects deserves comment. This component of sodium extrusion comprises passive permeability and transmembrane pathways not involving sodium/potassium-ATPase. At least one of these pathways sodium/lithium countertransport has been repeatedly reported as increased in essential hypertension (Hunt et al. 1986); further there is indirect experimental and epidemiological evidence that this abnormality reflects changes in erythrocyte lipids (Hunt et al. 1986). The overall
diminution in ouabain resistant pathways produced by linoleic acid in this study constitutes further evidence for the role of membrane lipid as a determinant of such fluxes.

The failure to observe a blood pressure lowering effect in the present study, despite my observations made in chapter 4 and other reports to this effect (Lorenz et al. 1983; Singer et al. 1984), may reflect the small number of subjects studied or may be due to variable absorption and incorporation of linoleic acid into the plasma membrane, which may obscure any effect upon blood pressure, however, the apparent contradictory changes in sodium efflux rates seen in both these studies is a largely unexplained finding which merits further study.

**DIETARY FAT MANIPULATIONS IN RAT GENETIC HYPERTENSION**

The results of this study demonstrate that, in the rat cell membrane, fatty acid profiles can be dramatically changed by dietary means without influence upon the development of blood pressure seen as animals age. A protective effect of the HLA or NLA diets on the development of hypertension in SHRs was not observed despite the large proportion of linoleic acid in the former and the high fat content of both diets. Wexler (1981) was able to prevent SHRs becoming hypertensive by feeding a high fat diet, but this diet was atherogenic in nature. Barcelli et al. (1982) have shown the experimental HLA diet, which was similar to the present one, increased renal PGE$_2$ synthesis when compared with the NLA control diet without any effect on blood pressure in Sprague Dawley rats fed such diets for 20 weeks.

Overall, erythrocyte and adipocyte lipid fatty acid profiles in this study reflected dietary fat intake. However in comparison
with the adipocyte, arachidonic acid was present in relatively large amounts in erythrocyte lipids and probably reflected desaturation and chain elongation of linoleic acid, either within the erythrocyte or prior to incorporation into this tissue. Such metabolism is not evident in adipocytes to the same extent, because this tissue is largely a lipid store. Between strain differences in lipid fatty acid profiles were found to exist in rats only if the high fat experimental diets were being given (HLA or NLA). These differences were present in erythrocytes, and although the WKY group was small, also in adipocytes. Such differences consisted of a highly significant rise in saturated fatty acids and an equally significant fall in linoleic acid in the SHR.

The presence of these abnormalities in membrane lipid composition in genetically hypertensive rats, if considered in the light of a reduced membrane fluidity found in these rats (Postnov et al. 1981; Orlov et al. 1982), and abnormalities in the activity of membrane bound enzymes, become very important and could reflect an underlying factor in disturbances of membrane function described in SHR. These changes in fatty acid profiles could not be corrected by changing the dietary P/S ratio because the abnormality in SHR was present if the rats were fed either the NLA or the HLA diet. What was interesting was that if a lower fat containing diet, such as the economy rat chow, was given no such abnormality could be detected in SHR suggesting that the quantity of dietary linoleic acid or total fat is important in bringing out this perturbation. Most if not all of the data suggesting a genetic defect in the plasma membrane of SHR has been obtained in rats fed economy rat chow with a similar
fat content to that in the present study. Thus the relationship between the abnormality demonstrated in SHR here, and other documented structural and functional membrane abnormalities is difficult to assess. However, adipocytes appear to share the abnormality in SHR erythrocyte lipid profiles when on higher fat diets suggesting the origin of the primary abnormality not to reside within cell plasma membrane itself, but possibly in the transport of dietary triacylglycerols in the blood, or perhaps even in the adsorption of fatty acids and glycerides across the gut.

Even in the absence of abnormalities of erythrocyte membrane lipid profiles, hypertension was still present in SHR, suggesting that the abnormality is not directly related to blood pressure in these animals but can be generated by increasing the amount of dietary fat. There are a large number of phenotypic differences between SHR and WKY animals. Therefore, it is possible, if not likely that differences in membrane handling of lipids are coincidental changes not directly linked to blood pressure. It is not possible to say whether an increase in dietary total fat or just linoleic acid is the stimulus for the emergence of this abnormality as the economy chow diet used in this study was found to contain more than twice the linoleic acid present in the NLA experimental diet.

ERYTHROCYTE MEMBRANE PHOSPHOINOSITIDE METABOLISM IN ESSENTIAL HYPERTENSION

These results demonstrate that $^{32}$P incorporation into phosphoinositides by the erythrocyte membrane is increased in the normotensive offspring of essential hypertensive patients, but unaltered in patients with raised blood pressure. There appeared
to be no association between the rate of $^{32}P$ incorporation and age; this implies that phosphoinositide metabolism proceeds at a higher rate of turnover in subjects genetically prone to essential hypertension when the pressure is normal, but that the turnover of these phospholipids is not different from control levels when increased blood pressure is established. Whilst abnormalities of phosphoinositide metabolism have been demonstrated in rat hypertension, these have been dependent on the age and strain of rat studied (Kiselev et al. 1981; Koutousov et al. 1983; Marche et al. 1985). The single study of $^{32}P$ incorporation in man reported that $^{32}P$ of Ptd Ins 4,5 was higher in patients with essential hypertension compared with control subjects whether the patients were on antihypertensive medication or not (Marche et al. 1985). However, the male to female ratio was different in the two groups and the results were not interpreted using analysis of variance. In the present study detailed consideration was given to the control groups to ensure close matching for age, sex, body weight and height, both for patients with hypertension and in their normotensive offspring. Whilst there is no evidence concerning the effect of these confounding variables upon phosphoinositide metabolism, such findings as weight and plasma lipids are related to other erythrocyte membrane functions such as ion transport (Roelofsen, 1981).

The finding of an abnormality of phosphoinositide metabolism in the erythrocytes of patients prone to the development of hypertension is intriguing. This tissue is not intimately associated with blood pressure homeostasis and the changes appear to be manifest only at a time when the blood pressure has yet to
rise. It would appear that there is a functional abnormality in
the plasma membrane of these cells from relatives of hypertensive
patients which has disappeared in later life.

The reason for the disappearance of this abnormality with
ageing is uncertain. One possible explanation may be related to
observed evidence for increased sympathetic nervous activity in
both man and the spontaneously hypertensive rat which also
disappears with ageing (Kawano et al. 1982; Goldstein, 1983).
However, it should be pointed out that there is no published
evidence for increased autonomic activity in normotensive
relatives. Since the phosphoinositide second messenger system
involved in some functions of the autonomic nervous system it is
possible that these changes are linked. It is also possible that
changes in the structure of membrane lipids influences both
functions (Bing et al. 1986), although in the absence of further
data such explanations must remain speculative.

PHOSPHOINOSITIDE METABOLISM IN RAT AORTA

These results demonstrate that phosphoinositide hydrolysis
is enhanced in young SHR but that this phenomenon disappears as
the animals age. Previous experiments have studied the kinetics
of \(^{32}\)P incorporation in erythrocyte inositol phospholipids in SHR
and Wistar rats (Kiselev et al. 1981). Unstimulated \(^{32}\)P
incorporation was lower in SHR at 4 weeks but the differences had
disappeared at 16 weeks. Conversely a more recent study suggested
reduced \(^{32}\)P incorporation into erythrocyte ghost phospho-
inositides at 3 and 15 weeks (Koutouzov et al. 1983). However,
the same authors have subsequently observed increased erythrocyte
phosphoinositide \(^{32}\)P incorporation in Sabra hypertension prone
animals compared with Sabra hypertension resistant rats when both
groups were fed a low salt diet, high salt diet or treated with
DOCA.

The current study indicates differences in inositide
hydrolysis in the SHR. In the young animals when blood pressure
was rising but not yet fully established $[^{3}H]$-inositol phosphate
accumulation was enhanced either unstimulated or in the presence
of noradrenaline. However, by 19 weeks when the hypertensive
process was fully established unstimulated inositol phosphate
accumulation was similar in the two groups of animals and
noradrenergic evoked responses were diminished in SHR, although
the $ED_{50}$ was not different in the two strains indicating no
differences in sensitivity to the agonist. Differences between
the two strains of animal are unlikely to be explained in terms
of smooth muscle hypertrophy in SHR because the results were
corrected on a tissue weight basis. However, this explanation
cannot be completely excluded as hypertrophy could be associated
with increased connective tissue or tissue water.

The difference in inositide metabolism in SHR is potentially
extremely important. At the time the blood pressure was rising in
young spontaneously hypertensive rats inositol phosphate
accumulation was enhanced, therefore, an increased basal turnover
of phosphoinositide lipids and a greater agonist-induced response
would be consistent with this cellular process being intimately
involved in events initiating the blood pressure rise. The
sympathetic nervous system is often implicated in the early phase
of hypertension. Certainly at 4 weeks there is evidence of
increased sympathetic activity (Yamori, 1976) and therefore in
vascular tissue enhanced phosphoinositide breakdown would be
expected. This would have two consequences: firstly increased
tone in vascular tissue in SHR, which has been demonstrated
(Hermsmeyer, 1976), and secondly an increased trophic stimulus on
the smooth muscle cells. In this context noradrenergic activity
has been shown to be a potent growth promoting factor in cultured
cells when the pressure load had been removed (Bevan, 1984) and
diacylglycerol generation from phosphatidylinositol metabolism
has been postulated as being instrumental in providing the
stimulus for cell division (Berridge, 1986). The findings in SHR
at 19 weeks suggest that the initial stimulus to raised blood
pressure was no longer present and in fact, not required; for by
then medial thickening would be sufficient to maintain the
hypertensive process.

Therefore this study demonstrates an abnormality in the
early stages of hypertension development, which is related to
membrane lipids and which involves messenger systems that are
important in blood pressure control by their influences on tone
and medial thickening. Further studies must now follow to induce
and reverse hypertension in animals whilst phospholipid
metabolism is followed.

**COARCTATION HYPERTENSION AND PHOSPHOINOSITIDE HYDROLYSIS IN THE
RAT**

These results demonstrate that the induction of coarctation
between the renal arteries of Wistar rats raised blood pressure
proximally and increased plasma renin concentration. Structurally
the aorta above the ligature demonstrated an increase in muscle
mass but no rise in nuclear density suggesting that the cellular
proliferative response observed was hypotrophy rather than
hyperplasia. Distal to the coarctation blood pressure fell but
in spite of this aortic structure only demonstrated small reductions in medial thickness and at 9 days mean cross-sectional area was actually increased. Above the constriction inositol phosphate production was non-significantly increased at 3 days but significantly so at 9 and 20 days: there was no change at any time below the coarctation. However, proximal hypertension was present at 72 hours. Plasma renin concentration was always greatly increased although it had begun to fall at 20 days; this was consistent with the almost complete destruction of the distal kidney noted in most animals at this time. The structural response induced in the proximal aorta was considered to be hypertrophic. Whilst this is in agreement with the findings of Olivetti et al. (1980) it contrasts with work in rabbits by Bevan et al. (1976) and in rats by Owens and Reidy (1985), whose techniques I employed for measurements of aortic dimensions. However, in these previous reports the coarctation induced was different: Bevan et al. placed the ligature proximal to the superior mesenteric artery, and Owens and Reidy used much older rats (5 months). In this latter experiment the constriction diameter was similar to the one used in the present study but in view of the much younger age of our animals (8-10 weeks) the coarctation would be more severe in the older rats. Inositol phosphate production was greater in distal aortic segments in both sham and in rats with coarctation, when compared with proximal tissue. Similar differences between proximal and distal aorta have been reported previously for sodium/potassium-ATPase activity and DNA synthesis (Owens and Reidy, 1985; Sugden et al. 1987); it has been shown that basal phosphatidylinositol hydrolysis influences aortic sodium/potassium-ATPase activity.
(Simmons et al. 1986), and there are undoubted links between inositol phospholipid metabolism and the early signals in the mitogenic response (Rozengurt, 1986). The differing levels of biochemical activity in different parts of the aorta are therefore consistent.

The significance of these results lies in the light thrown upon the involvement of phosphoinositide metabolism in cellular growth (Berridge 1987). The segments of aorta under test were dissected out of the animal and consequently away from endogenous stimuli for some hours before measurements were made; and yet in proximal aorta above the coarctation inositol phosphate production in the presence of lithium was increased at a time when structural alterations were proceeding. Accelerated hydrolysis of polyphosphoinositide lipids could herald an increase in nuclear activity (Rozengurt 1986): in accord with this Loeb et al. (1986) have recently demonstrated enhanced DNA synthesis in aortae from rats with two-kidney one clip renal hypertension at 3 days. At this time in our experiments rats had already demonstrated a rise in pressure proximal to the coarctation and this is a strong stimulus for structural alteration (Mulvany 1987).

This model also induces large rises in renin release due to ischaemia of the distal kidney. In turn, plasma angiotensin II concentrations will rise. It is known that this hormone activates the phosphoinositide system in vascular smooth muscle (Griendling et al. 1987), and may have trophic effects in addition to its vasoconstrictor properties (Lever 1986). Indeed, Plunkett and Overbeck (1985) have reported increased wall to lumen ratios in cremaster arterioles from rats with coarctation compared with
sham operated control animals. The authors postulate that such non pressure related alterations in structure might be attributable to systemic neural or humoral influences. In this experiment examination of the aorta distally did not reveal enhanced phosphoinositide hydrolysis suggesting that a humoral factor such as angiotensin II was not the sole mediator of the changes observed proximally. However, the blood pressures distal to the coarctation were 30-50% lower than those in sham operated rats, and yet there was little regression of aortic medial dimensions. Indeed at 9 days, cross sectional area was increased. In fact it was only at 20 days that some true structural regression was observed in the distal aorta, at a time when renin levels were in decline. These results therefore do not exclude a role for humoral factors such as angiotensin II in the trophic response to coarctation. Nevertheless, above the coarctation, it would appear most likely that the prime stimulus to growth was increased load. The exact mechanism by which this is transmitted into a proliferative response remains uncertain, but it is most likely due to stimulation of local mitogens which may then work through the phosphoinositide system (Berridge 1987). It is possible that the endothelial integrity may be important in this regard because distension or damage to this cell layer might initiate a growth response as a result of the injury allowing released mitogens access to exposed smooth muscle cells. Indeed it has been shown that removal of cultured endothelial cells from myocytes will provoke increased phosphoinositide hydrolysis in the presence of angiotensin II (Ganz et al. 1986).
The results of this study indicate the methods described are sensitive enough to demonstrate that stimulation of the phosphoinositide signalling system in resistance arterioles results in the production of inositol monophosphate, bisphosphate, 1,3,4 and 1,4,5 structural isomers of inositol trisphosphate and the highly phosphorylated Ins 1,3,4,5-P$_4$. This is probably the result of the stimulation of phospholipase C mediated hydrolysis of phosphoinositide as the production of inositol phosphate was associated with the concomitant reduction in radio-labelling of phosphoinositides.

Although it is not possible in the present study to state to what extent the hydrolysis of Ptd-Ins 4-P directly contributes to the very large increase in tissue Ins 1,4-P$_2$ observed, which may also be the result of dephosphorylation of inositol phosphates formed following hydrolysis of Ptd-Ins 4,5-P$_2$, however the highly significant increase in tissue Ins 1,3,4-P$_3$ and the very modest rise in Ins 1,3,4,5-P$_4$ which occur following the short period of stimulation provides evidence of the rapid formation and subsequent dephosphorylation of Ins 1,3,4,5-P$_4$. In addition these data indicate the loss of Ptd-Ins on stimulation, although this was not statistically significant, to be approximately three times greater than that gained by Ins 1-P. However, this may be the reflection of the rapid dephosphorylation of Ins 1-P to inositol catalysed by the lithium-sensitive inositol 1-phosphatase.

Because the period of stimulation in these experiments, carried out in the presence of labelled inositol, was terminated apparently before a period of phosphoinositide resynthesis, it is
unlikely that possible changes in isotopic specific activity associated with the various pools of inositol containing compounds would have any significant influence upon the observed agonist induced changes. Further work is required to determine the relative contribution of individual inositol phospholipid substrate to the agonist induced liberation of 1,2-DAG.

The detection of two inositol containing compounds in the region of the H.P.L.C. gradient corresponding to GroP-Ins 4,5-P₂ was exciting and unexpected but perhaps in accord with findings in other tissues (Downes et al. 1986; Hawkins et al. 1986) these two compounds were found probably not to represent esters of Ptd-Ins P₂ structural isomers but authentic Ptd-Ins 4,5-P₂ glycerol ester and an unknown inositol containing component eluting after GroP-Ins 4-P and just prior to GroP-Ins 4,5-P₂. This novel compound, which was resistant to periodate and hydrazine treatment, possibly indicating the absence of esterified glycerol, was shown to be sensitive to noradrenaline stimulation suggesting a possible role in phosphoinositide signalling. The period of pre-labelling was comparatively short in these experiments and it is unlikely that the existence of such an unidentified compound could be accounted for by the incorporation of radioactivity into a non-inositol containing compound. Upon hydrolysis one might expect the novel lipid soluble compound to release an inositol containing water soluble component. All radioactive peaks eluting in aqueous tissue extracts were identified as inositol phosphates which could originate from the successive dephosphorylation of Ins 1,3,4,5-P₄. Therefore it is possible that the novel hydrophilic product of hydrolysis is inositol or may co-elute with inositol phosphate. Alternatively, this component may be
more polar than Ins 1,3,4,5-P$_4$ and was undetected because the H.P.L.C. gradient of ammonium formate used did not exceed 1.7M.

More than two decades ago analysis of mammalian tissue lipid soluble inositol containing compounds revealed the presence of at least three phosphoinositides including one or more compounds with a suggested molecular structure closely resembling inositol containing lipid found in yeasts and the seeds of higher plans (Klenk and Hendricks, 1961; Ellis et al. 1963). More recently, the presence of a novel inositol containing lipid has been reported in cultured myocytes and in liver cell membranes by Saltiel et al. (1986). These workers have further shown this novel lipid to be rich in glucosamine and to be hydrolysed on exposure of tissue to hormone releasing a hydrophilic component capable of modulating the activity of cyclic AMP phosphodiesterase. It is becoming clear that interactions exist between cell second messenger systems (Yoshimasa et al. 1987). The novel compound found in rat resistance arterioles in the present study may represent such an interactive compound, more polar than the compound reported by Saltiel's group, but which nevertheless appears to be agonist-sensitive. It is of interest that despite an intense search two groups of workers have not detected such a compound to be present in lipid extracts prepared from rat parotid glands (Hawkins et al. 1986; Downes et al. 1986). I have been unable to find such a compound in rat brain lipid extracts and this may suggest a degree of tissue specificity. The novel compound detected here in resistance arterioles now requires further characterisation of structure and function.

The precise functions of individual inositol phosphates generated upon agonist stimulation of arterioles remain unclear.
However, the methods described here provide the means to investigate this further and indeed to extend studies into hypertension where a fundamental defect of this second messenger system may contribute to the increased vascular resistance observed in this condition.
12 CONCLUSIONS

A number of structural and functional abnormalities have been reported in essential hypertension and in rat genetic hypertension. Although such abnormalities are similar in both species, the majority of studies have been carried out in circulating blood cells whose role in the development of hypertension is unlikely to be of great importance. Also, such changes may be secondary to the increased blood pressure. However, the presence of similar changes in cells of normotensive offspring of hypertensives indicates a possible genetic association. Abnormalities of blood cell membrane ion transport mechanisms, membrane viscosity and lipid structure may reflect similar abnormal parameters in other tissues such as blood vessels which have a central role in blood pressure regulation. Indeed there is evidence with reference to sodium transport that this may be so (Aalkjaer et al. 1986).

Changes in the fatty acid composition of erythrocytes taken from human hypertensives, and the demonstration of similar findings in blood cells taken from SHR may provide an important link with other abnormalities of membrane structure. Such changes in the levels of membrane unsaturated fatty acids may contribute to the raised membrane viscosity in hypertension, and may be linked to changes in cellular cation handling, because ion pumps which are representative of active and passive movement processes depend upon the integrity of the cell plasma membrane. An alteration in the lipid environment may affect profoundly functional characteristics of such processes. It is of interest that a similar abnormality was found in the erythrocyte of SHR and essential hypertensive patients. However, this was not
detected in SHR unless the fat content of the diet was increased.

If the functional abnormalities of cells taken from hypertensive patients and SHR are secondary to a change in the membrane lipid environment, then a change in dietary fat which promoted changes in membrane lipids might be expected to alter functional membrane characteristics. In a group of normotensive human volunteers increasing the dietary intake of polyunsaturated fatty acids by 40 percent led to an increase in leucocyte sodium influx as assessed by the increase in sodium/potassium-ATPase activity. This was apparent within 4 weeks of commencing dietary treatment and was only associated with a significant fall in systolic blood pressure. In a smaller group of normotensive individuals who progressively increased their dietary intake of polyunsaturated fatty acids over a period of 56 days, it was not possible to detect a change in blood pressure, and in addition sodium/potassium-ATPase activity was not altered by the dietary change. However, passive sodium efflux from leucocytes and red cell membrane linoleic acid content was decreased. These studies indicate that in normotensive humans increasing the relative amount of membrane polyunsaturated fat results in a change in membrane function which may beneficially alter blood pressure. However, my experiments with n-6 fatty acids suggest that their effects on blood pressure and sodium transport are small: indeed the studies with increasing doses of linoleic acid suggest that the therapeutic usefulness of this manoeuvre may be limited. Studies with n-3 marine oils may hold more promise.

Rats with genetic hypertension possess abnormalities of blood cell sodium/potassium-ATPase. Cell membrane levels of linoleic acid were found to be decreased and associated with an
increase in the membrane content of non-polyunsaturated fatty acids. The levels of fatty acids in cell membranes of these animals could be altered profoundly by dietary means, however, if over a 13 week feeding period erythrocyte membrane lipids were enriched with linoleic acid by a two-fold increase this was shown to have no influence upon the development of hypertension in the SHR. This is in agreement with other experiments of this type and might reflect, for example, a strong trophic stimulus for the expression of hypertension in these animals which is very difficult to prevent after just a few weeks of age. Blood pressure development proceeded normally in control animals during the high polyunsaturated fatty acid feeding period and suggests this diet has no influence upon the development of blood pressure in either strain. Linoleic acid supplementation did have a very minor effect upon one component of blood pressure in normotensive humans in my original study, but not in a later dose ranging experiment, and therefore in this regard the experiments in SHR and man do have some similarity.

The difficulty in dissociating factors causing hypertension from those which are consequent to the high blood pressure is forever present in studies of this kind, and it becomes necessary to examine processes which may be abnormal in hypertension not only when the disease is fully established but at an early stage before hypertension develops. In human populations to obtain a group at the pre-hypertensive stage is difficult, and further, to predict accurately which individuals will develop hypertension in later life is almost impossible. In experimental animals such populations are not difficult to obtain. Hypertensive rats are inbred producing offspring with a high probability of developing
the disorder, although extrapolations from one species to another must be viewed with caution. In humans individual offspring of essential hypertensives have an unknown probability of becoming hypertensive but it is greater however than offspring of normotensive individuals. In my studies such a group of normotensive offspring of hypertensive individuals were found to have abnormal distributions of fatty acids in their erythrocyte membrane lipids. These differences were similar to changes found in hypertensive subjects, consisting of an increased proportion of oleic acid present, a change expected to reduce membrane fluidity. Other changes in membrane fatty acids present in hypertensives were not observed in normotensive offspring suggesting they may be secondary and possibly induced by the development of hypertension.

The metabolism of phosphoinositide lipids occurs in the cell plasma membrane and has become of prime interest in studies of tissue growth and reactivity as carried out in hypertension, and has been linked with the regulation of cation transport mechanisms. When phosphoinositide metabolism was investigated in the erythrocyte membrane of normotensive offspring of hypertensives it appeared to be overactive. The possibility that this signalling system is abnormal in the early stages of development of hypertension is supported by observations in segments of aorta taken from young SHR before hypertension was established where the production of inositol phosphate was increased. Therefore this important signalling system is overactive in human and rat genetic hypertension and furthermore in blood vessels in the SHR, a tissue closely associated with blood pressure regulation. In performing these experiments in
mature SHR and in humans where hypertension was established, quite consistent results have been obtained. When hypertension is fully established in the human erythrocyte, no abnormality of phosphoinositide metabolism was detected; similarly no such abnormality was detected in the aorta of adult SHR in the absence of agonist, and when noradrenaline was applied a reduced response was found. From these data it is apparent that age or perhaps blood pressure dependent changes to membrane structure and function were being observed in both species and it becomes interesting to speculate that the alteration in erythrocyte membrane lipid fatty acid composition seen in normotensive humans genetically predisposed to hypertension is closely associated with changes in phosphoinositide metabolism. In pre-hypertensive individuals in the presence of an increase in membrane oleic acid, phosphoinositide metabolism is overactive. However, in hypertensives where abnormalities in membrane lipid fatty acids are more widespread, phosphoinositide metabolism is normal. Thus the membrane lipid environment may be the regulatory factor, but in the absence of further studies, cause and effect cannot be assumed. Indeed to be acceptable, such lipid mechanisms would require to some extent, selective re-routing of dietary lipids from incorporation into membrane lipids to fat stores and there is evidence for this as adipose tissue fatty acids have been shown to correlate well with hypertension in human populations, and in my experiments in rat genetic hypertension SHR receiving an identical diet to that of control rats had large differences in adipose fatty acid profiles.

To further investigate the role of the phosphoinositide signalling system in the development of raised blood pressure,
inositol phospholipid hydrolysis was studied in vascular tissue in coarctation hypertension. This model of induced hypertension proved useful as genetic influences were not a factor in the rapid development of hypertension proximal to the aortic ligature. Distal to the ligature the animal was hypotensive, and high circulating levels of angiotensin II enabled the investigation of two parameters believed to play a part in the development of genetic hypertension, circulating vasoconstrictor agents and increased load on the blood vessel wall. Data obtained from this experiment indicated dramatic early changes in aortic characteristics proximal to the ligature as phosphoinositide metabolism, media thickness and vessel size were all increased compared with controls. Nuclei counts indicated the growth response to be hypertrophic in nature and all changes paralleled the increase in blood pressure. Below the coarctation there was evidence that the high circulating level of angiotensin II in the presence of a reduced haemodynamic pressure had little or no effect upon phosphoinositide metabolism and aortic atrophy was found towards the end of the experiment, suggesting that either increased blood pressure alone, or increased pressure and circulating vasoconstrictive hormone is required to increase basal phosphoinositide metabolism and associated increases in smooth muscle growth.

Studies of the phosphoinositide signalling system in large capacitance blood vessels such as the aorta are only of limited value in a disease such as hypertension as these vessels contribute nothing to peripheral vascular resistance. Smaller resistance blood vessels whose size are of less than 500μm in internal diameter are more relevant. Also, the measurement of
gross inositol phosphate accumulation does not provide information of the levels of second messengers such as Ins 1,4,5 P$_3$ which is a minor proportion of total cellular inositol phosphate. As the level of inositol phosphate present in small resistance arterioles is much lower than in large segments of aorta, a high performance liquid chromatographic method was used to achieve a high resolution separation of minor inositol phosphates and their structural isomers and to concentrate these peaks into one or two fractions of column eluate for scintillation counting. This equipment was used to investigate this second messenger system in resistance arterioles taken from Wistar rats and enabled the identification of five inositol phosphates. These were present in similar proportions to other tissues and the calcium releasing second messenger Ins 1,4,5 P$_3$ was found to represent a very small proportion of the total inositol phosphate present. When resistance arterioles were stimulated using noradrenaline, inositol phosphates changed dramatically from the basal profile, resulting in a rise in all five inositol phosphates. Analysis of membrane bound inositol lipids indicated the presence of four inositol containing peaks of which one was found not to contain glycerol and at the present time remains unidentified. All lipid associated inositol containing components were reduced during noradrenaline stimulation and suggests this method of analysis may be useful in future investigations to investigate basal stimulated inositol phosphates and their precursor lipids in resistance arterioles in human and experimental hypertension.

In conclusion, my experiments have demonstrated that the membrane lipid environment of cells from hypertensive patients
and their normotensive first-degree offspring is abnormal; similar disturbances were present in genetically hypertension-prone rats but only manifested by diets rich in linoleic acid compared with normal rat chow. Attempts at lowering blood pressure by dietary supplementation with linoleic acid failed in spontaneously hypertensive rats and after small falls in man with a modest addition of linoleic acid this was not confirmed using larger doses. An examination of phosphoinositol metabolism in rats and man suggested that the phospholipids are abnormally overactive in the early phases of the development of hypertension. Thus, I have provided evidence that membrane lipid content may be contributing at least in part, to the initial processes that lead to the elevation of pressure. This appears to be confirmed by the results of my studies of vascular phosphoinositide metabolism during the development of hypertension in rats with coarctation of the aorta, although an examination of this lipid system in resistance vessels in human hypertension remains to be carried out.
BIBLIOGRAPHY


ALLAN D, MICHELL RH (1979), The relationship between Ca^{2+} mediated polyphosphoinositide phosphodiesterase activity, 1,2-diacylglycerol accumulation and microvesiculation in erythrocytes. Prog Clin Biol Res 30:523-529.


BOLTON TB (1979), Mechanism of action of transmitters and other substances on smooth muscle. Physiol Revs 59,606-718.

BORISKINA GM, GOULAK PV, POSTNOV YuV (1978), Phosphoinositide content in the erythrocyte membrane of rats with spontaneous and renal hypertension. Experientia 34,744.

BRIGHT R (1836), Tabular view of the morbid appearances in 100 cases connected with albuminous urine. With observations. Guy's Hospital Reports 1,380-400.


CODDE JP (1985), The effects of varying dietary polyunsaturated fat intake on tissue prostaglandin synthesis and blood pressure regulation in rats (PhD thesis, Department of Medicine, University of Western Australia, Perth, Western Australia) 65-88.


de WARDENER HE, MACGREGOR GA (1980), Dahl's hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure: its possible role in essential hypertension. Kidney International 18,1-9.

de WARDENER HE, MACGREGOR GA (1982), The natriuretic hormone
and essential hypertension. Lancet 1,1450-1454.


DONALDSON AN (1926), The relation of protein foods to hypertension. California and West Coast Medicine 24,328-331.

DOWNES CP, HAWKINS PT, IRVINE RF (1986), Inositol 1,3,4,5-tetrakisphosphate and not phosphatidylinositol 3,4-bisphosphate is the probable precursor of inositol 1,3,4-trisphosphate in agonist-stimulated parotid gland. Biochem J 238,501-506.


FRIEDMAN SM, NAKASHIMA M, MCINDOE RA, friedman CL (1976), Increased erythrocyte permeability to Li and Na in spontaneously hypertensive rats. Experientia 32,476.


GARWITZ ET, JONES AW (1982), Aldosterone infusion in the rat and dose-dependent changes in blood pressure and arterial ionic transport. Hypertension 4,374-381.


GRYGLEWSKI RJ, BUNTING S, MONCADA S, FLOWER RJ, VANE RJ, VANE JR (1976), Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. Prostaglandins 12,685.

GULAK PV, BORISKNA GM, POSTNOV YuV (1972), Ca-binding to the
outer surface of red blood cell membranes in spontaneously hypertensive rats and essential hypertensive patients. Experientia 379,191-195.


HADDY F, PAMNANI MB, SWINDALL BT, JOHNSTON J, CRAGOE EJ (1985), Sodium channel blockers are vasodilator as well as natriuretic and diuretic agents. Hypertension 7 (suppl 1) 121-126.

HAMBERG M, SVENSSON J, SAMUELSSON B (1975), Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proc Natl Acad Sci USA 72,2994.


HAWKINS PT, STEPHENS L, DOWNES CP (1986), Rapid formation of inositol 1,3,4,5-tetrakisphosphate and inositol 1,3,4-trisphosphate in rat parotid glands may both result indirectly from receptor-stimulated release of inositol 1,4,5-trisphosphate from phosphatidylinositol 4,5-bisphosphate. Biochem J 238,507-516.


IAcono JM, DOUGHERTY RM, PUSKA P (1982), Reduction of blood pressure associated with dietary polyunsaturated fat. Hypertension 4 (suppl III), 34-42.


IMA I A, GERSHENGORN MC (1986), Phosphatidylinositol 4,5-bisphosphate turnover is transient while phosphatidylinositol turnover is persistent in thyrotropin releasing hormone-stimulated rat pituitary cells. Proc Natl Acad Sci USA 83, 8540-8544.

IRVINE RF, ANGGARD EE, LETCHER AJ, DOWNES CP (1985), Metabolism of inositol 1,4,5-trisphosphate and inositol 1,3,4-trisphosphate in rat parotid glands. Biochem J 229, 505-511.

IRVINE RF, MOOR RM (1986), Microinjection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent upon external Ca2+. Biochem J 240, 917-920.

IZZARD AS, HEAGERTY AM (1988), The measurement of internal pH in resistance arterioles: evidence that intracellular pH is more alkaline in SHR than WKY animals. J Hypertension (in press).


JOHNSON G (1868), On certain points on the anatomy and pathology of Bright's disease of the kidney. II. On the influence of the minute blood vessels upon the circulation. Transactions of the Royal medical and Chirurgical Society 51, 57-78.


KATO H, TAKENAWA T (1987), Phospholipase C activation and diacylglycerol kinase inactivation lead to an increase in diacylglycerol content in spontaneously hypertensive rat. Biochem Biophys Res Commun 146,1419-1424.


KIMELBERG HG, PAPHADJOPOULS D (1972),Phospholipid requirements
for (Na\(^{+}K^{+}\))-ATPase activity: head-group specificity and fatty acid fluidity. Biochim Biophys Acta 282,277-292.


LOCKETTE W, OTSUKA Y (1986), Phorbol ester, vascular relaxation, and cGMP. Hypertension 8,827.


MIALL WE and OLDHAM PD (1963), The arterial factor in arterial blood pressure. Br Med J 1,75.


MICHELL RH (1975), Inositol phospholipids and cell surface receptor function. Biochim Biophys Acta 415,81-147.


MINNEMAN KP, ABEL PW (1984), 'Spare' alpha, adrenergic receptors and the potency of agonists in rat vas deferens.


PEATTIE ME, BUSS DH, LINDSAY DG, SMART GA (1983), Reorganisation of the British total diet study for monitoring food constituents from May 1981. Food and Chemical Toxicology 21,503-507.
PICKERING GW (1968), High blood pressure. Edinburgh: Churchill-Livingstone.


POSTNOV YuV, GULAK PV, ORLOV SN (1986), Evidence for alteration of the phosphorylation of the erythrocyte membrane skeleton in rats with spontaneous hypertension (SHR) J Hypertension 4 (suppl 6) s367-s369.


POSTNOV YuV, ORLOV SN, POKUDIN NI (1979), Decrease of calcium binding by the red blood cell membrane in spontaneously hypertensive rats and in essential hypertension. Pflugers Archives 379,191-195.


RAAB W, FRIEDMAN R (1936), The condition of blood circulation of vegetarians. Z Klinishe Medische 130,505.


ROUSE IL, BEILIN LJ, ARMSTRONG BK, VANDONGEN R (1984), Vegetarian


SAILE F (1930), Influence of vegetarian food on blood pressure. Medishe Klinishe 26,929-931.


SAILE F (1930), Influence of vegetarian food on blood pressure. Medishe Klinishe 26,929-931.


SELYE H, STONE H (1946), Pathogenesis of the cardiovascular and renal changes which usually accompany malignant hypertension. J Urol 56,399-419.


SINGER P, JAEGGER W, VOIGT S, THIEL H (1984), Defective desaturation and elongation of N-6 and N-3 fatty acids in hypertensive patients. Prostaglandins Leukotrienes and


STRECKER RB, HUBBARD WC, MICHELAKIS AM (1975), Dissociation constant of the norepinephrine receptor complex in normotensive and hypertensive rats. Circ Res 37,658-663.


SUGDEN AL, BEAN BL, STRAW JA (1987), Effects of high potassium or low sodium diet on vascular Na\(^{+}\),K\(^{+}\)-ATPase activity and blood pressure in young spontaneously hypertensive rats. Hypertension 9,571-575.


SWALES JD (1983), Abnormal ion transport by cell membranes in hypertension; In Handbook of Hypertension, Volume 1, Amsterdam, Elsevier Press.

SWALES JD, TANGE JD (1970), Photo-electric method of blood pressure measurement in the rat. J Lab Clin Med 75, 879-885.


TOKUMURA A, MOSTAFA MH, NELSON DR, HANAHAN DJ (1985), Stimulation of (Ca²⁺+Mg²⁺)-ATPase activity in human erythrocyte membranes by synthetic lysophosphatidic acids and lysophatidylcholines. Effects of chain length and degree of unsaturation of the fatty acid groups. Biochim Biophys Acta 812, 568-574.


WILSON DB, CONNOLLY TM, BROSS TE, MAJERUS PW, SHERMAN TE, TYLER AN, RUBIN LJ, BROWN JE (1985), Isolation and characterisation of the inositol cyclic phosphate products of polyphosphoinositide cleavage by phospholipase C:
In these studies the structure and metabolism of cell membrane lipids was investigated during the development of human hypertension and in animal models of this disease.

Erythrocyte membrane fatty acid profiles were found to be similarly deranged in genetic hypertension prone rats, in human hypertensive patients and their normotensive offspring. Such profiles were found to be readily manipulated in normotensive human volunteers by changes in dietary fat intake; increasing dietary polyunsaturated fat caused a fall in blood pressure and altered membrane sodium handling.

In genetically hypertension prone rats polyunsaturated fat induced increases in cell membrane linoleic acid content but did not prevent the development of hypertension suggesting that the amount and the type of fat may be important in blood pressure amelioration.

Basal cellular phosphoinositide metabolism was increased in the erythrocytes of normotensive offspring of human hypertensives and in the aortae of young hypertension prone rats. This was not detected in either species in established hypertension. Reduced agonist-induced responses were found in vascular tissue from adult genetically hypertensive rats.

It was concluded that in essential hypertension and rat genetic hypertension alterations in plasma membrane fatty acid profiles may underlie abnormalities of membrane function. Increased phosphoinositide metabolism found as hypertension was developing may be a genetically determined mechanism initiating changes to vascular structure and blood pressure.

In the rat, coarctation hypertension increased basal vascular phosphoinositide metabolism and the development of aortic smooth muscle cell hypertrophy paralleled the increase in blood pressure, no changes were seen in aorta exposed to reduced pressure. It is concluded that vascular hypertrophy was induced through phosphoinositide metabolism stimulated by increased blood pressure. This may be important in maintaining increased peripheral vascular resistance seen in hypertension.