STUDIES OF ERYTHROCYTE SODIUM

TRANSPORT IN ESSENTIAL HYPERTENSION

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This thesis is dedicated to my family, my wife Teresa, my daughters Lulie and Leyla and to the memory of my parents.
Preface

Essential hypertension is the best known risk factor for cardiovascular disease and mortality. It afflicts approximately 10% of the population and despite intensive research, the cause for this condition remains unknown. The only consistent abnormality in established essential hypertension is a raised total peripheral vascular resistance but research into the underlying mechanisms has been frustrated by the difficulties in obtaining these small vessels for study in man. One possible mechanism may involve an alteration in cell membrane sodium handling in vascular smooth muscle and the difficulties in obtaining living arterial tissue has led many workers to use more readily available cells such as fat or circulating blood cells.

This thesis reports my studies of erythrocyte sodium handling in patients with essential hypertension and their normotensive offspring compared to normotensive subjects with no family history of hypertension. It deals with the processes involved in sodium movements across cell membranes, and the effects of dietary manipulation such as change in salt intake and increasing the intake of potassium or of polyunsaturated fat on erythrocyte sodium transport and blood pressure.
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CHAPTER 1

SODIUM TRANSPORT AND ESSENTIAL HYPERTENSION
Introduction

Most studies of patients with essential hypertension have shown no abnormality of total exchangeable sodium (Tarazi 1976; Birkenhager and Schalekamp 1976; Schalekamp and Birkenhager 1977) or extracellular fluid volume (Novak, Strong and Hunt 1972; Ibsen and Leth 1973; Tarazi 1976). In the earliest phase of essential hypertension, cardiac output is often elevated (Werko and Lagerlof 1949; Conway 1968; Frohlich, Tarazi and Dustan 1969; Sannerstedt 1966) but plasma volume is normal (Frohlich, Tarazi and Dustan 1971). Furthermore, plasma volume is reduced in patients with higher blood pressure levels (i.e. $\geq 105$ mm Hg diastolic blood pressure) and the extent of the reduction is directly correlated with the height of the diastolic blood pressure (Frohlich, Tarazi and Dustan 1971; Tarazi 1976; Messerli, De Carvalho, Christie et al. 1978; Bing and Smith 1981).

Thus, there is no evidence that sodium retention or plasma volume expansion is responsible for the raised blood pressure in essential hypertension.

However, there remains the possibility of an alteration in the distribution of sodium and water within the body tissues without a change in external sodium balance. Tobian and
Binion (1952) first reported an increase in sodium and water content in post mortem specimens of renal artery and psoas muscle taken from patients who had had essential hypertension. Although, these studies related to larger vessels, Tobian suggested that similar changes could occur in the arterioles, causing a reduction in luminal diameter and a rise in peripheral resistance. Later (1954) these workers demonstrated similar changes in the aortic wall of rats with renal and deoxycorticosterone salt hypertension. These vessels showed similar sodium retention and furthermore the sodium content fell after the hypertension was reversed by removal of the renal artery clip (Tobian, Janecek, Tomboulian et al 1961). It was postulated that the "water logging" would bring about increased thickening and stiffness of peripheral vessels and explain the raised total peripheral resistance in essential hypertension. This work was criticised on several grounds: firstly, post-mortem changes may have caused the changes observed in the human tissue and secondly, the changes could equally well become a consequence of hypertrophy rather than the cause of the hypertension. In addition, none of the vessels studied contribute significantly to the peripheral resistance and no animal model of essential hypertension had been studied.
Since, for the most part, actual measurements could be made only on large arteries and there was considerable difficulty in obtaining living arterial tissue from patients with untreated essential hypertension, many workers tried more readily available cells such as adipocytes or circulating blood cells. Until recent times, it had to be assumed that changes observed in these cells reflect what is happening in the vascular smooth muscle of the resistance vessels. However, Aalkjaer, Heagerty, Parvin et al (1986) compared sodium efflux rates in leucocytes and human mesenteric vessels in omental fat taken from subjects undergoing laparotomy and demonstrated a significant correlation between the two, although efflux of sodium from the mesenteric vessels was many times faster.

Much of the work on sodium movements in and out of cells in essential hypertension has been performed using the erythrocyte and has produced many conflicting results. However, before considering the data obtained from studies of the erythrocyte, it is important to examine the mechanisms which underlie sodium movements in these cells and the factors which influence these systems.
Erythrocyte Ion Transport

There is a higher concentration of potassium (the major intracellular cation) inside the cell than in the extracellular fluid and the opposite is true for sodium (the major extracellular cation). Clearly, this distribution is far from an equilibrium, and differs from that observed with anions which follow the distribution predicted by the Gibbs-Donnan equilibrium across the cell membrane. For some time, this peculiar distribution was attributed to certain impermeable properties of the cell membrane to cations. However, in the 1930s it was shown that cold storage of blood caused erythrocytes to lose potassium and accumulate sodium. Moreover, when cold stored erythrocytes were re-incubated at body temperature the cells re-accumulated potassium and lost sodium. In 1941, Harris observed that reversal of the cold induced changes depended upon the presence of plasma and could be prevented by agents which inhibit glucose metabolism.

There is now ample evidence to show that cations leak into or out of cells along their respective electrochemical gradients and that an active process moves these cations back uphill against their gradients. The energy for this process comes from the breakdown of adenosine triphosphate
(ATP). The enzyme responsible for the hydrolysis of ATP, adenosine triphosphatase and which requires both (Na)$^+$ and (K)$^+$ for its activity was first demonstrated in crab nerve axon (Skou 1957) and 4 years later it was demonstrated in disrupted red cell membranes (Dunham and Glynn 1961). Furthermore the inhibition of this enzyme activity by the cardiac glycoside or strophanthin (ouabain) also inhibited active (Na)$^+$ and (K)$^+$ transport in intact erythrocytes (Schatzmann 1953; Khan and Acheson 1955; Joyce and Weatherall 1955; Solomon, Gill and Gold 1956; Glynn 1957; Post and Jolly 1957; Gill and Solomon 1959; Glynn 1969).

The active extrusion of sodium is dependent on the external potassium concentration and the active outward movement of sodium is coupled with active inward movement of potassium (Steinbach 1952; Glynn 1954). Thus, in intact red cells this system requires both external potassium and internal sodium for its function (Whittam 1962; Glynn 1962). Sodium affinity is higher on the inside of the membrane than outside and vice-versa for potassium. Under physiological conditions the hydrolysis of one ATP molecule is associated with the extrusion of 3 sodium ions and the entry of 2 potassium ions (Glynn 1962). The unequal stoichiometry of the pump indicates that it is electrogenic and contributes
Fig 1: The ion transmembrane transport processes in the erythrocyte. (1 - Active NaK ATPase pump, 2 - Na - Na countertransport, 3 - NaK co-transport).
to the membrane potential of the cell, but its main function is to maintain the electrochemical gradients on which the membrane potential depends.

Whilst it is clear that passive diffusion and the ouabain sensitive sodium pump play a major role in determining intracellular concentration of sodium and potassium, these ions may move across the plasma membrane by other protein carrier mediated systems (Fig 1).

**Exchange diffusion**

This was defined by Dunn (1970) as an equal flux of a single ion species in both directions across the plasma membrane, mediated in each case by the same carrier and not therefore inducing net transport.

**Counter transport**

This is the movement of an ion in one direction coupled to the movement of the same ion species in the opposite direction across the cell membrane (Garrahan & Glynn 1967 a & b). For sodium it is measured by using lithium. Erythrocytes are loaded with lithium and the efflux of lithium from the cells is measured in the presence and absence of external sodium (Canessa, Adragna, Solomon et al
1980; Tosteson, Adragna, Bize et al 1981). This mechanism is inhibited by phloretin (Duhn, Gobel, Lorenz et al 1982). Since exchange takes place on a one to one basis, if the transport system operates in vivo only in this mode it could not affect intracellular sodium content.

**Cotransport**

This is the movement of an ion species in one direction coupled to the movement of a different ion species in the same direction. It has been known for many years that in the presence of ouabain the remaining ionic movements cannot be explained on the basis of diffusion alone. Tracer studies have demonstrated that an unidirectional sodium (Na\(^{+}\)) and potassium (K\(^{+}\)) influx exhibited a large saturable component (Glynn 1957). These ouabain resistant influxes were mutually dependent so that removing one ion from the medium inhibited the saturable component of the influx of the other ion (Wiley and Cooper 1974). Later studies showed that these ionic movements were inhibited by diuretics such as ethacrynic acid and frusemide (Lubowitz and Whittam 1969; Dunn 1970; Wiley and Cooper 1974).

Sodium potassium co-transport has been measured as potassium stimulated \(^{22}\text{Na}^{+}\) influx and sodium stimulated \(^{42}\text{K}^{+}\) efflux (Wiley and Cooper 1974) or by the efflux of these
ions from sodium loaded erythrocytes in the presence of ouabain (Garay, Dagher, Pernollet et al 1980a). In addition to inhibition by diuretics, sodium potassium cotransport is dependent on the presence of the chloride ion (Chipperfield 1980).

If the rate of transport in both directions was equal, there would be no net effect on intracellular cation content. A net inflow of sodium has been described by Wiley and Cooper (1974). However the net movement may be quite small (Cumberbatch and Morgan 1983) or non-existent (Brand and Whittam 1983; 1984).

Factors which influence erythrocyte sodium handling

Several studies have reported that erythrocyte sodium content is dependent upon age, sex and race. Age related changes appeared to be more marked in females (Beilin, Knight, Munro-Faure et al 1966), but where wide age groups were studied, intracellular sodium was higher in the 40 to 70 year old males and females, compared to a 20 to 39 year old groups (Funder and Wieth 1966b). More recent studies by Naylor (1970) and Naylor, Dick, Worrall et al (1977) have confirmed a lower sodium content in young compared to old females, but they observed no such change in men. Furthermore, Naylor and his colleagues (1977) also reported
that active erythrocyte sodium transport decreased with age and passive permeability increased. Sodium potassium ATPase activity also fell with age and more recently similar changes have been demonstrated in aging males (Gambert and Duthie 1983). Weissberg, West and Woods (1983) confirmed that intracellular sodium was lower in young females compared to young males and both groups were well matched for age, weight and height. Other studies have failed to observe a sex difference in erythrocyte intracellular sodium (Love and Burch 1953; Valberg, Holt, Paulson et al 1965; Czaczkes, Ullmann, Ullmann et al 1963). However these workers used small groups of subjects and age matching was ignored or it was not documented. Sex matching also appears to be important in studies of erythrocyte sodium cotransport and countertransport systems since both are reduced in women compared to men (Duhm, Gobel, Lorenz et al 1982; Williams, Hunt, Kuida et al 1983).

Racial background also has a considerable influence on erythrocyte sodium content. Thus, Love and Burch (1953) noted high erythrocyte sodium levels in negro blood donors and later this finding was confirmed by Munro-Paure, Hill and Anderson (1971). Furthermore, ouabain sensitive 86 rubidium uptake is reduced in blacks (Woods, Beevers and West 1981a) and another study of ethnically distinct populations showed that sodium-potassium ATPase activity was
reduced in Asians, blacks and Jewish white subjects compared
to Scandinavian white subjects (Beutler, Kruhl and Sacks
observations by showing that erythrocyte sodium was elevated
in blacks and males compared to whites and females. In
addition they noted a significant inverse correlation
between sodium-potassium ATPase activity and erythrocyte
sodium concentrations. There are also racial differences in
sodium influx (Etkin, Mahoney, Forsthoefel et al 1982) and
Na-Li counter transport (Trevisan, Cooper and Semos 1983).
One study has examined all aspects of erythrocyte sodium
handling with respect to ethnic origin (M'Buyamba Kabangu,
Lijnen, Groeseneken et al 1984). They observed that
intracellular sodium was raised, Na-Li countertransport and
rubidium uptake are decreased in association with enhanced
sodium influx in negros. Furthermore, the same study
reported a reduction of frusenide-resistant sodium efflux
(cotransport) and this finding has been confirmed by workers
in America and France (Garay, Hannaert, Dagher et al 1981;

Erythrocyte sodium handling can be altered in many disease
states and by a number of hormones (Cumberbatch and Morgan
1981; Parker and Berkowitz, 1983). Thus it is well
established that genetically determined haematological
disorders, for example hereditary spherocytosis, are
associated with an elevated intracellular sodium and increased sodium-potassium ATPase activity (Wiley 1972). In sickle cell disease, both patients (homozygotes and heterozygote carriers) exhibit reduced erythrocyte Na-K cotransport and elevated sodium content (Crook and Mroczkowski 1985). Duchenne muscular dystrophy is associated with reduced Na\(^+\)/K\(^+\) ATPase activity and active sodium extrusion (Souweine, Bernard, Lasne et al 1978).

Erythrocyte sodium concentration is altered in myocardial infarction and congestive heart failure (D'Amico 1958), hypokalaemia (Astrup 1974), digoxin toxicity (Wessels, Samizadeh and Losse 1974) and haemorrhagic shock (Cunningham, Wagner and Shires 1970). Other conditions may be associated with abnormalities of membrane sodium handling: thus uremic plasma inhibits ouabain sensitive ATPase activity (Cole, Balfe and Welt 1968) and in patients with chronic renal failure, erythrocyte membrane ouabain-sensitive ATPase is decreased (Cole 1973). Alteration in the activity of Na\(^+\)/K\(^+\) ATPase also has been observed in hyperthyroidism (Smith and Samuel 1970; Cole and Waddell 1975) and Cushing syndrome (Wambach, Helber, Bonner et al 1980).
Since many hypertensive patients are overweight, the effects of obesity must also be taken into account. Two studies by De Luise, Blackburn and Flier (1980) and Flier, Usher and De Luise (1983) have reported a reduction in erythrocyte pump sites, decreased ouabain sensitive sodium pump activity and a raised intracellular sodium. However, Mir, Charalambous, Morgan et al (1981) showed that erythrocyte sodium-potassium ATPase activity and ouabain sensitive sodium efflux were both significantly elevated in obese subjects. The discrepancy between these studies may well depend on the use of groups of mixed racial background since Beutler, Kruhl and Sacks (1983) reported that there were no differences in sodium transport of obese subjects when they were divided into ethnically distinct populations.

The influence of antihypertensive therapy must be considered since a number of studies of sodium transport in hypertension have involved treated patients. In the leucocyte, diuretic therapy returned a depressed sodium pump activity to normal (Poston, Jones, Richardson et al 1981b) and lowered intracellular sodium (Araoye, Khatri, Yao et al 1978). Similarly when the normotensive relatives of hypertensive patients were treated with diuretics for 7 days, leucocyte active sodium efflux rose but blood pressure was unchanged (Milner, Heagerty, Bing et al 1984). By
contrast, diuretic treatment caused a rise in erythrocyte sodium (Walter 1981). More recent studies with calcium antagonists have revealed discrepant results. Nifedipine lowered blood pressure but failed to alter $\text{Na}^+$ transport (Heagerty, Bing, Thurston et al. 1983), but verapamil was reported to produce a similar effect to diuretics (Gray, Poston, Hilton et al. 1984).

**Essential Hypertension**

**The Erythrocyte**

Losse, Wehmeyer and Wessels (1960) were the first group to report an elevation of intracellular sodium in erythrocytes obtained from patients with essential hypertension. Since then there have been over twenty reports of erythrocyte sodium content in essential hypertension. The early finding was confirmed by von Gessler (1962); Wessels, Junge-Hülsing and Losse (1967) and more recently by Fadeke Aderoumu and Salako (1979); Urry, Trapane, Andrews et al. (1980); Poston, Sewell, Wilkinson et al. (1981a); Clegg, Morgan and Davidson (1982); Birks and Langlois (1982). However, many other workers have failed to detect any difference in intracellular sodium in hypertensive patients (Weller 1959; Bracharz, Laas and Betzein 1962; Schroeder 1968; Burch 1971; Munro-Faure, Hill and Anderson 1971; Canessa, Adragna,

There are several possible explanations for this discrepancy. In some studies, ethnic influences were not considered. For example, Fadeke Aderoumu and Salako (1979) studied black Africans and Poston, Sewell, Wilkinson et al (1981a) used hypertensive patients of mixed racial background. In others, there are problems with age and sex matching of the control subjects. Also many workers also have paid little attention to the potential influence of previous anti-hypertensive therapy. Interestingly, the two early studies of Losse, Wehmeyer and Wessels (1960) and von Gessler (1962) and the later one of Clegg, Morgan and Davidson (1982) used untreated hypertensive subjects.

Family history may be another important influence. Clegg, Morgan and Davidson (1982) found that erythrocyte sodium was raised in hypertensive patients but the highest values were to be found in subjects with a family history of hypertension.

Losse and his co-workers followed up their original observation by demonstrating an increased $^{22}\text{Na}$
accumulation in the erythrocytes of hypertensive patients (Wessels, Junge-Hulsing and Losse 1967). The net trans-membrane flux measured by this technique represents the sum of passive diffusion and carrier mediated processes affecting sodium movements across the cell membrane. Increased passive sodium influx would be the simplest explanation but alternatively, a carrier mediated exchange mechanism could be activated (Canessa, Adragna, Solomon et al 1980). Postnov, Orlov, Shevchenko et al (1977); Etkin, Mahoney and Forsthoefel et al(1982) and Birks and Langlois (1982) also reported increased passive permeability to sodium in hypertensive patients.

Increased erythrocyte membrane permeability to sodium and the consequent rise in the intracellular sodium concentration would normally stimulate sodium efflux via the energy dependent ouabain sensitive sodium pump. Postnov, Orlov, Shevchenko et al (1977) observed that the Na\(^+\)/K\(^+\) ATPase pump was stimulated and ouabain sensitive \(^{86}\)rubidium uptake has also been reported to be increased (Woods, Beevers and West, 1981a). Fadeko-Aderoumu and Salako (1979) showed that the erythrocyte sodium efflux rate was not significantly lower in hypertensive patients. Another group reported increased sodium efflux rate constant (that is the fraction of intracellular sodium extruded in unit time) only in cells incubated in plasma of hypertensive
patients (Fitzgibbon, Morgan and Myers 1980). Similarly, Wambach and Helber (1981) found that Na\(^+/K^+\) ATPase activity was increased in the erythrocyte. However, Swarts, Bonting, de Pont et al (1981) detected no difference at all and Poston, Sewell, Wilkinson et al (1981a) found a reduction in erythrocyte sodium efflux rate constant with a non-significant elevation of absolute sodium efflux rate. Walter and Distler (1982) found that the ouabain sensitive sodium efflux rate constant was decreased but since the intracellular sodium was higher, the absolute ouabain sensitive efflux was unchanged in hypertensive patients. Since the majority of the reported red cell studies are based on measurements of cation transport in vitro, it is possible that such measurements may not reflect events in vivo. Thus, cation transport may be altered by the procedures involved in separating the cells from whole blood or by the artificial perturbation of cell physiology used in some techniques. Recently, Boon, Aronson, Hallis et al (1984) described a method for studying cation transport in vivo by comparing the accumulation of rubidium in erythrocytes with the plasma levels after the oral administration of rubidium chloride. They found that in vivo cation transport measured by this technique was enhanced in untreated hypertensives compared to age matched control subjects (Boon, Aronson, Hallis et al 1986). Thus, the majority of reports suggest that erythrocyte sodium
efflux is enhanced in essential hypertension because of stimulation of the sodium-potassium ATPase pump per se since the number of pump sites is not changed (Smith, Owen-Ash, Hunt et al 1984; Boon, Harper, Aronson et al 1985).

Two other carrier mediated transport pathways have been investigated in essential hypertension and found to be abnormal. Prusenide sensitive sodium-potassium cotransport measured as the rate of sodium to potassium extrusion in sodium loaded ouabain-pretreated erythrocytes has been reported to be decreased in patients with essential hypertension (Garay, El Ghozi, Dagher et al 1980b). Remarkably, there was no overlap between the values of the essential and secondary hypertensive subjects and it was proposed that this was a genetic marker which could be used as a diagnostic test for essential hypertension.

Others have confirmed a decrease in sodium potassium cotransport (Garay, Hannaert, Dagher et al 1981; Davidson, Opie and Keding 1982; Tuck, Gross, Maxwell et al 1984; Montanari, Sani, Canali et al 1984; but in all these series there was considerable overlap between hypertensive and control subjects. Two studies have failed to show any difference (Swarts, Bonting, De Pont et al 1981; Wiley, Clarke, Bonacquisto et al 1984) but in both many of the hypertensive subjects were studied whilst receiving
anti-hypertensive therapy. Finally, a later joint study by Garay and co-workers indicated that sodium-potassium cotransport was actually increased in American hypertensive subjects (Canessa, Bize, Solomon et al 1981).

Sodium-sodium countertransport is measured by pre-loading erythrocytes with lithium and comparing its extrusion into a medium containing a physiological concentration of sodium or no sodium (Canessa, Adragna, Solomon et al 1980). The resulting difference probably represents the activity of a carrier mediated system which exchanges erythrocyte and extracellular sodium on a 1:1 basis. Sodium-lithium countertransport has been reported to be raised in essential hypertension (Canessa, Adragna, Solomon et al 1980; Cusi, Barlassina, Ferrandi et al 1981; Canali, Borghi, Sani et al 1981; Clegg, Morgan and Davidson 1982; Ibsen, Jensen, Wieth et al 1982; Trevisan, Cooper and Sempos 1983; Brugnara, Corrocher, Foroni et al 1983; Montanari, Sani, Canali et al 1984) and in only two papers it was found to be normal (Swarts, Bonting, De Pont et al 1981; Duim, Gobel, Lorenz et al 1982). However, throughout the literature there is a strong tendency for this abnormality to be most pronounced in patients with a positive family history of hypertension. Thus, when the two negative studies were re-analysed to take
the influence of family history into account, they supported
the general view that lithium efflux is elevated in
essential hypertension (Morgan, Stewart and Davidson 1986).

In conclusion although there have been problems relating to
patient selection, mismatching for sex, age, race and the
confounding effects of exposure to anti-hypertensive drugs,
one can conclude that influx of sodium into the erythrocyte
is enhanced in essential hypertension. Possibly as a
consequence, absolute sodium efflux is increased but in
addition sodium-sodium countertransport is also increased
and sodium-potassium cotransport is reduced. The precise
significance of the changes in the latter two systems
remains unclear since neither has a significant influence on
net sodium movements across the cell membrane.

White Cell Sodium Transport

Although they are technically more difficult, studies of
white blood cell sodium handling may perhaps more closely
reflect any disturbances of cation ion transport in vascular
smooth muscle cells. For this reason some workers have
studied sodium movements in lymphocytes or a mixed leucocyte
population. Studies with these cells have considerable
advantages: they are more active and like the smooth muscle
cell are nucleated. Unlike the erythrocyte, the leucocyte
can synthesise protein and contains over 10,000 sodium pump sites in its plasma membrane compared to only 300 in the erythrocyte. On the other hand, isolation is more difficult and fewer cells can be obtained for study.

The first study of leucocyte sodium transport in essential hypertensives reported an increased intracellular sodium in association with a reduced efflux rate constant (Edmondson, Thomas, Hilton et al 1975). Unfortunately, this study was flawed because sodium content was measured at room temperature but sodium efflux rate constant was determined with the cells incubated at 37°C. Moreover it is difficult to accept the authors' statement that absolute efflux rate could not be calculated, but using their data it would again appear that total sodium efflux rate is elevated in essential hypertension. Araoye, Khatri, Yao et al (1978) confirmed that intracellular sodium was raised in a mixed leucocyte population, but the poor sensitivity of the method was reflected by a wide scatter of individual values. Another study of lymphocytes also demonstrated an increase in intracellular sodium which was directly correlated with the blood pressure (Ambrosioni, Tartagni, Montebugnoli et al 1979). More recently, Poston, Sewell, Wilkinson et al (1981a) investigated leucocyte sodium transport in essential hypertension but utilised a mixed group of treated and untreated patients of different racial backgrounds.
Intracellular sodium was markedly raised to more than twice that of the controls and the sodium efflux rate constant was depressed. Heagerty, Milner, Bing et al (1982) also examined sodium handling in leucocytes and found a minor reduction in sodium efflux rate constant which was largely accounted for by a decrease of the ouabain sensitive sodium pump activity. Careful matching of the white Caucasian hypertensive patients to the controls avoided the pit falls of the previous studies. Interestingly, the technique for measuring intracellular sodium was much refined producing less scatter but a very definite increase in sodium compared to the controls. Taking all the white cell data together, it is clear that sodium efflux rate constant is depressed but the absolute sodium pumping appears to be increased. Despite the technical difficulties these few reports do confirm the findings in the erythrocytes of an increased intracellular sodium and absolute sodium pumping in essential hypertension.

**Normotensive Offspring of Hypertensive Patients**

Many of the studies in essential hypertension have been repeated using the normotensive offspring of patients with essential hypertension and similar abnormalities have been observed. Erythrocyte intracellular sodium has been reported to be raised in normotensive first degree relatives.
of hypertensives (Henningsen, Mattson, Norstin et al. 1979; Gudmundsson, Berglund, Herlitz et al. 1983), and total sodium efflux rate was found to be elevated by Cooper, Miller, Trevisan et al. (1983). However, others report no change (Gudmundsson, 1984a; Cusi, Barlassina, Ferrandi et al. 1981) or a decrease (Lijnen, M'Buyamba-Kabangu, Fagard et al. 1984). Others have suggested that sodium pump activity is increased after finding increased $^{86}$rubidium uptake (Woods, Beevers and West 1981b).

Passive sodium influx was found to be elevated by Henningsen, Mattson, Norstin et al. (1979), but this was not confirmed by Gudmundsson, Herlitz, Jonsson et al. (1984b). Studies of sodium-potassium cotransport indicate a decrease (Meyer, Garay, Nazaret et al. 1981; Cusi, Barlassina, Ferrandi et al. 1981; Lijnen, M'Buyamba-Kabangu, Fagard et al. 1984) and Na-Li countertransport is increased in the offspring of patients with essential hypertension (Woods, Falk, Pitman et al. 1981).

The leucocyte shows a slightly different pattern of change with a raised intracellular sodium but a decreased ouabain sensitive sodium efflux rate constant (Heagerty, Milner, Bing et al. 1982). However, the changes in membrane sodium
handling in the erythrocyte and the leucocyte of offspring of essential hypertensives resemble those observed in the same cells of patients with essential hypertension.

**Genetic and Experimental Hypertension**

Almost all of the investigations of sodium handling by vascular smooth muscle have been carried out in the rat. The majority of these studies have utilised genetic hypertension in an inbred strain in the belief that this may provide a better model for essential hypertension than models which involve renal manipulations or mineralocorticoid administration. Animal studies have considerable advantages: it is possible to examine cation transport processes before hypertension develops and in addition they permit direct access to blood vessels. However, great care must be taken in the extrapolation from the animal models to man.

In the spontaneously hypertensive rat (SHR), most studies of erythrocyte (Postnov, Orlov, Gulak et al 1976; Friedman and Friedman 1976) and arterial wall (Jones 1974; Friedman 1979; Friedman, McIndoe and Spiekerman 1982) show that sodium and potassium permeability is increased and associated with increased turnover of these cations. There are two reports of experiments using thymocytes (equivalent to the
leucocyte) in spontaneously hypertensive rats. One reported an elevated intracellular sodium and reduction in ouabain sensitive sodium efflux rate constant and claimed that these changes are correlated with the blood pressure (Jones, Patrick and Hilton 1981). However, no account of age was made and when this was considered, the association was lost and the absolute sodium efflux appeared to be enhanced (Bradlaugh, Heagerty, Bing et al 1984). Vascular smooth muscle intracellular sodium content has not been observed to be raised in the SHR (Friedman, Nakashima, Friedman 1975; Aalkjaer, Kjeldsen, Norgaard et al 1985).

Similar findings have been demonstrated in mineralocorticoid (Friedman, Nakashima,Friedman 1975) and renal artery clip or kidney wrapping hypertension (Brock, Smith and Overbeck 1982; Overbeck and Crisette 1982). The stimulus to enhanced sodium efflux is probably increased sodium influx and this has been demonstrated in DOCA hypertension (Jones, 1981). These changes are not the result of the raised blood pressure per se since these findings were confirmed using aortic tissue from above and below an aortic constriction in aldosterone treated rats (Overbeck and Crisette 1982).

Most of these studies have been carried out with tissues maintained in artificial media and it is possible that plasma constituents could create differences in vascular
cation turnover. Only one group has studied isolated arterial wall sodium pump activity (measured by ouabain sensitive rubidium uptake) with plasma included in the incubation medium (Pamnani, Buggy, Huot et al 1981). Under these conditions, sodium pump activity was reduced in mineralocorticoid hypertension, but curiously the ouabain sensitive pump activity in the salt sensitive Dahl rat measured in this way was increased (Pamnani, Clough, Huot et al 1980; Overbeck, Ku and Rapp 1981).

In summary, the majority of studies have shown that both the spontaneously hypertensive and mineralocorticoid hypertensive rats have enhanced sodium influx and efflux rates and thus parallel the findings in human hypertension.

Relationship between sodium transport abnormalities and blood pressure elevation

Despite the problems created by errors in laboratory methods and matching of controls, all the reviewed evidence points to a link between abnormalities of cell membrane handling of sodium and hypertension. Such an association could be explained in one of two ways: the electrolyte changes may be a consequence of hypertension or alternatively the process underlying the disturbance of ion transport could participate in the mechanism responsible for the elevated
pressure. It is unlikely that these changes are the result of hypertension since the abnormalities are present in circulating blood cells not obviously involved in the hypertensive process. Moreover, similar changes can be demonstrated in normotensive relatives of hypertensive subjects (Meyer, Garay, Nazaret et al 1981; Heagerty, Milner, Bing et al 1982) but not in patients with renovascular hypertension (Garay, Elghozi, Dagher et al 1980b; Birks and Langlois 1982).

The alternative view that the sodium transport abnormalities are somehow related to the cause of hypertension is therefore more likely. However, the blood pressure elevation may not be necessarily a direct consequence of these changes. Thus the sodium flux abnormality may participate directly in smooth muscle vasoconstriction or could act merely as a marker for a global disturbance in cell membrane function which also gives rise to hypertension by another process.

The hypothesis that the cation abnormalities are directly involved in producing enhanced smooth muscle contractility was formally proposed by Blaustein (1977). He suggested that there is a linked countertransport system in erythrocytes and vascular smooth muscle which exchanges calcium for sodium ions. This system is inhibited by a
raised intracellular sodium and so calcium efflux from the cell is reduced. Consequently, intracellular calcium increases and smooth muscle cell contractility is enhanced.

When intracellular ionised calcium concentration lies within a critical range on the dose response contraction curve, he calculated that smooth muscle tension would be dependent upon intracellular sodium. Thus, using representative values for electrolyte concentration, he calculated that an increase of 5% in intracellular sodium will increase smooth muscle tension by 50%. A circulating ouabain-like inhibitor of the sodium pump such as that postulated by Haddy, Pannani and Clough (1978) could therefore increase peripheral resistance through an elevation of intracellular sodium, inhibiting sodium-calcium exchange and thereby raising intracellular calcium concentration.

De Wardener and MacGregor (1980) developed this hypothesis to include human essential hypertension by postulating that the kidney possessed a genetic defect which impaired salt excretion. They argued that this was aggravated throughout life by too much salt in the diet: the resulting sodium overload leading to expansion of the blood volume. In response to this, the hypothalamus secretes a hormone with ouabain like activity which promotes renal sodium excretion. In addition, this inhibitor causes widespread depression of
the Na⁺/K⁺ATPase pump. The resulting rise in intracellular sodium would according to Blaustein (1977) lead to a rise in intracellular calcium subsequent to interference of the sodium:calcium exchange mechanisms. A rise in intracellular calcium would increase vascular smooth muscle tone and raise peripheral vascular resistance. It has been suggested that there would be a rise in blood pressure. The rate and degree of the hypertension depending upon the severity of the inherited deficit in sodium excretion and the dietary salt intake. The hypothesis of de Wardener and MacGregor (1980) is supported only by a minority of studies which showed reduced ouabain sensitive sodium efflux rate constants in blood cells of patients with essential hypertension. Furthermore, the defect in leucocyte sodium transport is more severe in patients in whom the renin-angiotensin system fails to show a normal response to salt restriction (Edmondson and MacGregor 1981). In further studies, incubating normal leucocytes in serum from patients with essential hypertension it was shown the defect in ouabain sensitive sodium efflux rate constant present in leucocytes of hypertensive patients could be induced in cells from normotensive subjects (Poston, Sewell, Wilkinson et al 1981a) and indirect measurement of Na⁺/K⁺ ATPase inhibitor activity in the plasma of hypertensives (de Wardener, MacGregor, Clarkson et al 1981) have provided support for the putative sodium transport
inhibitor. However, despite an intensive search by many workers, the "natriuretic factor" has been neither identified or isolated: but there are indications of a low molecular weight substance which binds to digoxin antibodies in the plasma of patients with primary hypertension (Hamlyn, Ringel, Schaeffer et al 1982) and \( \text{Na}^+/\text{K}^+ \) ATPase inhibitors have been found in the plasma of normotensives with a family history of hypertension (Devynck, Pernellet, Cloix et al 1984).

However, the de Wardener and MacGregor (1980) hypothesis does not account for the findings in the offspring of hypertensive subjects or the multitude of other membrane defects observed such as sodium-lithium countertransport, sodium-potassium cotransport, sodium influx and abnormalities of calcium binding found in patients with essential hypertension. Another difficulty lies in the failure to demonstrate any evidence of volume expansion either during the development or after the establishment of essential hypertension (Tarazi 1976; Beretta-Piccoli and Weidman 1984; Lund-Johansen 1983). Indeed the majority of studies demonstrate that both plasma volume and extracellular volume are reduced as the diastolic blood pressure rises (Tarazi 1976; Messerli, De Carvalho, Christie et al 1978; Bing and Smith 1981).
The absence of evidence for blood volume expansion in either early or established essential hypertension led de Wardener and MacGregor (1982) to modify their hypothesis and argue that central cardio-pulmonary volume diversion is the primary event. Even so if one accepts this explanation it is hard to see how this mechanism could be involved in the pathogenesis of hypertension. Central cardio-pulmonary blood volume diversion could result in a selective increase in efferent sympathetic nerve activity or a decrease in pulmonary venous compliance. The former would seem most unlikely and the latter would not raise cardiac output or blood pressure. Thus, the stimulus for the secretion of the ouabain inhibitory factor appears to be absent or reduced in both early and established essential hypertension.

The alternative hypothesis proposes that there is a genetic abnormality in the physicochemical structure of the cell plasma membrane underlying the alterations in a variety of electrolyte transport mechanisms, membrane calcium handling and blood pressure (Swales 1982; Orlov and Postnov 1982; Heagerty, Riozzi, Brand et al 1985). A decrease in plasma membrane calcium binding is the most common manifestation of the membrane defect in essential hypertension in erythrocytes (Postnov, Orlov and Pokudin 1979; Postnov and Orlov 1980), adipose tissue (Postnov and Orlov 1980) and smooth muscle (Wei, Janis and Daniel 1976).
In addition erythrocyte membrane viscosity is increased (Orlov and Postnov 1982).

Thus, if increased intracellular calcium because of decreased cell membrane affinity for calcium is responsible for enhanced vascular smooth muscle contractility, then the described changes in potassium and sodium handling could merely represent a marker for the primary abnormality. It is also a possibility, however, that these are more directly linked to calcium handling. Two properties of calcium may be relevant. Firstly, increasing intracellular calcium enhances erythrocyte sodium and potassium permeability (Romero and Whittam 1971) and there is a close relationship between potassium loss from erythrocyte ghosts and intracellular calcium (Whittam 1968; Romero 1976). Secondly, a high external calcium concentration stabilises the smooth muscle cell membrane, reducing potassium efflux and inhibiting excitation (Rothstein 1968; Holloway and Bohr 1973). Jones (1974) suggested that the increased turnover of potassium in arterial smooth muscle in spontaneously hypertensive rats (SHR) and deoxycorticosterone (DOC) salt hypertension (Jones and Hart 1975) is due to a decreased ability of cell membranes in these models to bind calcium. Furthermore, changes in calcium affinity involves the inner side of the cell membrane composed predominantly of the negatively-charged phospholipids, which constitute an
essential requirement for the sodium pump (de Caldentey and Wheeler 1979).

A primary abnormality of the phospholipid component of the cell membrane could therefore underlie both the decreased membrane calcium affinity and its consequences, together with perturbations of sodium pumping observed in some tissues. Alternatively the disturbance in cell membrane fluidity could impair calcium pumping through the Ca$^{2+}$ATPase in analogous fashion to inhibition of sodium pumping.

There is good evidence for decreased calcium binding by the cell membranes in spontaneously hypertensive rats and perhaps also in essential hypertension. There are theoretically two mechanisms by which this would induce changes in membrane handling of sodium and potassium. Either increased intracellular calcium concentration would increase permeability to these ions or decreased binding of calcium would destabilise the membrane, producing the same end results. In either case a link would be forged between the well documented increase in cell permeability to sodium and potassium, and vasoconstriction which would result from an increase in free intracellular calcium.
Thus the sequence of changes according to the membrane abnormality hypothesis would involve a global disturbance of membrane function causing an elevation of intracellular calcium and reduced sodium pump activity; this would give rise to a secondary increase in sodium and potassium permeability with increased intracellular sodium tending to oppose primary reduction in sodium pumping.

The studies described in this thesis were designed to test the hypothesis of de Wardener and MacGregor by examining sodium movements in hypertensive patients and normotensive offspring and observing the effects of manipulating sodium balance, potassium balance and dietary fat intake on blood pressure and sodium transport.
CHAPTER 2

METHODS
Subjects and Patients

All the subjects taking part in these studies gave informed consent and the protocols were approved by the Leicester Local Ethical Committee.

Hypertensive Patients:

Patients with essential hypertension all had a diastolic blood pressure greater than 90 mm Hg on at least two occasions, had never received therapy and had no evidence of a secondary cause for their raised blood pressure.

Normotensive Control Subjects:

These were recruited from the general population and had a diastolic blood pressure of less than 90 mm Hg on screening. They were selected prospectively according to whether there was a family history of hypertension or not. Where information was inadequate, first degree relatives were asked to visit either the Department of Medicine or their general practitioner to have their blood pressure measured. A positive family history was defined as having at least one parent or sibling with hypertension or having died from a complication attributable to hypertension.
Physiological Measurements

On entering a study all subjects had their height, weight (Marsden's weighing machines, London) and blood pressure (Hawksley Random Zero Sphygmomanometer) measured. Blood pressure and pulse were determined supine and upright and the mean of three readings taken. In the dietary intervention studies, the normotensive subjects were asked to provide three 24 hour urine samples to determine dietary sodium and potassium intake. A blood sample was taken to measure plasma renin activity (PRA) and erythrocyte electrolyte content and sodium transport. In the dietary fat study (Chapter 6) erythrocyte fatty acid composition was determined using a fresh 10 ml sample of heparinised blood.

Laboratory methods

The erythrocyte was chosen for study because it offers several advantages over other cells for the study of electrolyte content and membrane transport. It was the first cell to be studied and the processes which regulate the entry and exit of substances across the membrane are well known. In addition, erythrocytes can be readily and repeatedly obtained from both patients and normotensive subjects. In all these studies, erythrocytes were separated
from freshly drawn blood samples taken into lithium heparin vacutainers (Becton-Dickinson Ltd).

**Erythrocyte Electrolyte Composition**

In the past four decades there have been many publications on the determination of sodium and potassium content of human red cells. Surprisingly, although both of these electrolytes can be measured readily and with considerable accuracy by flame photometry, the literature reveals a wide scatter of normal values (Houtsmuller 1959; Valberg, Holt, Paulson et al 1965; Sieberth 1971). These discrepancies led Funder and Wieth (1966a) to review all the methods employed and evaluate the sources of error influencing red cell sodium and potassium measurement. Flame photometry was reliable and accurate with a coefficient of variation of 2% for potassium and 3% for sodium. The modern flame photometer with internal lithium standards had reduced the variations to less than 1%. By contrast, the isolation of red cells from plasma was beset with a multitude of pitfalls.
These include:

1. Change in plasma pH
2. Variation in the composition because of different age of red cells.
3. Leucocyte contamination.
4. Plasma trapping

Changes in plasma pH during isolation of erythrocytes:

Water content and hence red cell volume varies with plasma pH. This depends on the Gibbs-Donnan effect of non-diffusible ions (haemoglobin and organic phosphates) which are the main negative change inside the erythrocyte. When plasma pH falls, these groups bind hydrogen ions and the electrical charge inside the cell decreases. Electrical neutrality is maintained by the rapid uptake of chloride and bicarbonate ions from plasma and slowly sodium and potassium are lost from the cell. The net result is a rise in intracellular chloride and bicarbonate, water is taken up and erythrocyte volume increases. The converse applies when plasma pH rises. Funder and Wieth (1966a) showed that plasma pH rose by 0.5 unit and cell water content fell by 1.5-3.0% when blood was centrifuged in an open tube. The simple expedient of using capped tubes reduced the pH change
to 0.002 unit which would have negligible effect on red cell volume.

**Variations of electrolyte content due to cell age**

In vivo labelling with $^{59}$Fe has revealed that erythrocytes sediment differentially according to age. Thus, the top of the haemocrit column contains young (less dense) and the bottom old (more dense) cells (Chalfin 1956 and Prankerd 1958). Early studies reported dramatic differences in electrolyte content with young cells being reported as having more (Keitel, Berman, Jones et al 1955) or less (Bernstein 1959) sodium than older cells. However, Borun (1963) and Funder and Wieth (1966a) both reported that sodium content was increased in the older cells at the bottom of the haemocrit column. In addition, potassium and water content was decreased in old cells. Consequently, differences in erythrocyte sodium content are particularly marked when expressed in terms of red cell solids. Funder and Wieth (1966a) found that red cell sodium content varied by 7% and potassium by 10% depending on whether cells were taken from the top or bottom of the haemocrit column.
Leucocyte contamination

Approximately 90% of leucocytes are found in the buffy coat after centrifugation. Thus, removal of this layer effectively removes the major portion of leucocytes in blood samples (Funder and Wieth 1966a).

Trapping of plasma between erythrocytes

Studies with tracers suggest a wide range of plasma trapping in the erythrocyte column of 5-10% after centrifugation at 1700 g. This decreases to 3% with centrifugation at 15,000g (Burch 1971). Various methods have been utilised to estimate or overcome plasma trapping and these involve using (a) a fluid correction, (b) radioactive tracers, (c) washing to remove trapped plasma.

(a) Errors due to subtracting a fixed correction for plasma trapping. This will contribute a major error in erythrocyte sodium determination. When sodium is determined in unwashed red cells, a 1% error in the estimation of plasma trapping would yield a 20% error in intracellular sodium content. In order to keep the error in erythrocyte sodium content to 1% or less plasma trapping needs to be
known to an accuracy of 0.1% (based on a red cell sodium of 7 mmol and plasma sodium of 140 mmol Burch 1971). Since fixed plasma trapping corrections would be subject to errors which are considerably greater than 1%, this method is not acceptable.

(b) Errors in estimating plasma trapping, using radioactive tracers: Various tracer substances have been used to measure "trapped" plasma such as inulin, Evans blue and radioactive labelled albumin, sucrose, EDTA and sodium\(^{22}\). To produce reliable results the tracer must not penetrate red cells or be adsorbed on the surface. Maizels and Remington (1959a&b) found that the amount of extracellular fluid or suspending medium trapped in centrifuged erythrocytes depended upon the marker used. High molecular weight markers such as I\(^{131}\) albumin yielded appreciably lower values than lower molecular substances such as inulin or \(^{22}\)sodium. These findings were confirmed by Nagaki and Teraoka (1977) who found that high molecular weight markers gave high erythrocyte sodium values of 9.8 mmol/l of red cells compared to lower molecular weight markers results of 7.7 mmol/l. It follows therefore that "trapped" extracellular material should be described as "trapped albumin" or "trapped sodium". Brender and Wieth (1966a) showed that "trapped" albumin was 1.05 ± .01% compared to trapped sodium\(^{22}\) of 3.19±.02%. This 2-2.5%
difference in the estimation of trapped plasma was confirmed by Sieberth (1971) who also demonstrated that the difference between low and high molecular weight markers increases with increasing centrifugal forces. He suggested that the large molecules were forced out from the gaps between the erythrocytes.

(c) Washing erythrocytes to remove trapped plasma:
A number of workers have utilised washing with isotonic "sodium free" solutions to eliminate the sodium error due to plasma trapping. Choline chloride (Marunghiu, Holtmeier, von Klein-Wisenber 1966) or magnesium chloride (Smith 1972) both remove 99.5% of trapped plasma after three washes. However, sodium may be lost from the erythrocytes and cell volume might change during the washing process. Valberg Holt, Paulson et al (1965) observed a fall in sodium when erythrocytes were washed with magnesium chloride but this was not confirmed by subsequent studies (Nagaki and Teraoka 1977). Astrup (1974) found a small 0.9 mmol/kg fall in intracellular potassium with washing and by analogy assumed that loss of sodium would be less because of lower concentration gradient. Furthermore, they found that the third and final wash with magnesium chloride did not contain sodium.
Normal values for erythrocyte electrolyte content

By controlling the factors outlined in the preceding section, Funder and Wieth (1966b) then went on to provide values for sodium, potassium and water content for normal erythrocytes. No correction was made for plasma trapping because they were of the opinion that the correction for trapping was well within the error of erythrocyte electrolyte determination.

The results showed a high potassium (89.6 mmol/kg red cells, 134.6 mmol/kg water or 268.4 mmol/kg solids) and a low sodium (10.9 mmol/kg red cells, 16.5 mmol/kg water or 32.8 mmol/kg solids) Water content was 666 G/kg of red cells or 1995 G/kg of solids. There was a strong correlation between the sum of sodium and potassium concentration and water content supporting the view that the regulation of cell volume is closely related to the cation content. Red cell sodium was lower in young (<39 years) compared to older (>40 years) subjects. Although Funder and Wieth did not correct for plasma trapping, they did determine \(^{22}\text{Na}\) trapping and calculated that this reduced intracellular sodium by 1.9 mmol/kg red cells when plasma sodium was within the physiological range. Since 1965, a number of
workers have applied similar critical methods to determinate
red cell sodium (Nagaki and Teraoka 1977) and the normal
values obtained are compared in Table 1.

Funder and Wieth (1966a) related erythrocyte electrolyte
content to cell weight by weighing small aliquots of packed
cells using a balance accurate to 100 ug. This technique
was modified by later workers using the oxyhaemoglobin
measurement and allowed electrolyte values to be related to
a litre of packed erythrocytes. This method involves packing
erthrocytes by centrifugation at 2400 g for 30 minutes in a
closed conical tube. Aliquots of 0.1 ml were then drawn off
using an opsonic pipette, lysed with 7mM NH₄OH and the
haemolysate made up to 50 ml (Wooton 1964). Electrolyte
composition per litre of cells was calculated using the
optical density of packed cells and the density of the
haemolysate used for flame photometry.

After reviewing the methods currently available, it was
clear that electrolyte content was most reliably related to
erthrocyte dry weight of solids rather than packed cell
volume (Funder and Wieth, 1966a). It was decided therefore
to use erythrocytes washed with 99 mmol MgCl₂ to eliminate
trapped sodium and relate the electrolyte content to
erythrocyte dry weight.
Table 1 - Comparison of normal values for red blood cell sodium and potassium concentrations from the recent literature. (Nagaki and Teraoka 1977)

<table>
<thead>
<tr>
<th>Marker for measurement of trapped plasma</th>
<th>Number of subjects</th>
<th>Sodium mmol/L of cells</th>
<th>Potassium mmol/L of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evans Blue *</td>
<td>50</td>
<td>9.71</td>
<td>101.7</td>
</tr>
<tr>
<td>131I-albumin</td>
<td>128</td>
<td>10.56</td>
<td>-</td>
</tr>
<tr>
<td>131I-albumin</td>
<td>51</td>
<td>8.79±0.98</td>
<td>101.3±4.0</td>
</tr>
<tr>
<td>Evans Blue *</td>
<td>570</td>
<td>10.2±1.27</td>
<td>98.84±2.37</td>
</tr>
<tr>
<td>22Na +</td>
<td>128</td>
<td>7.37</td>
<td>-</td>
</tr>
<tr>
<td>14C-sucrose +</td>
<td>48</td>
<td>7.85</td>
<td>99.8</td>
</tr>
<tr>
<td>14C-sucrose +</td>
<td>51</td>
<td>6.90±0.77</td>
<td>102.5±4.06</td>
</tr>
<tr>
<td>Co-EDTA +</td>
<td>30</td>
<td>8.82</td>
<td>91.7</td>
</tr>
<tr>
<td>Washing method</td>
<td>159</td>
<td>7.35±1.26</td>
<td>99.1±5.3</td>
</tr>
</tbody>
</table>

* Markers of large molecular size
+ Markers of small molecular size
Measurement of Erythrocyte Electrolyte Content

A 10 ml freshly drawn blood sample was centrifuged at 2000 g in cool spin MSE centrifuge at room temperature for 10 minutes in a capped tube. The plasma was removed and stored at 4°C for later use in the sodium efflux measurements. The red cell pellet was washed twice with ice cold magnesium chloride (99mmol/l) and recentrifuged at 4°C. After a second wash, 2 x 500 ul of red cell pellet was removed from the middle of the column and each mixed with 1000 ul of deionised water in a Sarstedt tube. Complete mixing was achieved by gentle shaking. Three 100ul aliquots of haemolysate were transferred from each Sarstedt tube into pre-weighed aluminium foil sacks (approximate weight 15 mg). The sacks were dried to constant weight in an oven at 100°C for 24 hours. Each sack was then reweighed and the electrolyte contents leached out into either 50 ul concentrated nitric acid or 1 ml deionised water over 4-5 days. Since electrolyte recovery was the same with either nitric acid or deionised water, later experiments were performed on triplicates leached out into deionised water alone. Electrolyte leaching was completed by 4-5 days, after which each sample was centrifuged at 2000 rpm at room temperature for 20 minutes. 450 ul of supernatant was transferred to a plastic tube containing 450 ul of 30 mmol/l
lithium chloride and mixed on a Whirlimixer. A 450 uL aliquot of this solution was then further diluted 1:2 by the addition of 450 uL of 15 mmol/l lithium chloride. Sodium and potassium concentration was measured using a Corning flame photometer with an internal lithium standard. A blank estimation was performed using empty aluminium foil sacks leached in deionised water and the reading subtracted from each sample.

**Calculation**

The direct readings of the flame photometer less the blank readings were multiplied by the dilution factor (x 4) and divided by 200 (the dilution of the flame photometer standards) to give electrolyte concentration in mmol/litre of leaching deionised water. The result was divided by the weight of the red cell pellet in mgms and multiplied by 1000 mg to give a concentration of sodium or potassium in mmol/kg dry weight of red cells.

**Intra-assay variation of erythrocyte electrolyte concentration**

Blood samples taken into two separate vacutainers, from eleven subjects were used to assess the intra-assay variation of intracellular sodium and potassium measurement.
On each occasion three red cell haemolysate pellets were used to determine the electrolyte content of two samples of washed erythrocytes.

The coefficient of variation was calculated using

\[ CV = \frac{SD}{\bar{X}} \]

where SD is the pooled standard deviation (obtained by analysis of variance) and \( \bar{X} \) is the pooled mean of both studies.

**Results**

The individual values and the mean ± SEM for the two samples are shown in Table 2. The pooled mean for intracellular sodium was 21.6 mmol/kg dry weight with a pooled standard deviation of the individual values of 1.21 giving a coefficient of variation of 5.6%. Intracellular potassium was 225.4 mmol/kg dry weight with a pooled standard deviation of 12.3 and giving a coefficient of variation of 5.4%.
Table 2 - Intra-assay variation of erythrocyte electrolyte concentration

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>Sample 1</th>
<th></th>
<th>Sample 2</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>[Na] i</td>
<td>[K] i</td>
<td>[Na] i</td>
<td>[K] i</td>
</tr>
<tr>
<td>1</td>
<td>26.3</td>
<td>233</td>
<td>23.2</td>
<td>239</td>
</tr>
<tr>
<td>2</td>
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<td>3</td>
<td>21.1</td>
<td>218</td>
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<td>5</td>
<td>16.7</td>
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<td>16.7</td>
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<td>6</td>
<td>21.1</td>
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<td>23.8</td>
<td>202</td>
</tr>
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<td>7</td>
<td>22.2</td>
<td>254</td>
<td>21.3</td>
<td>240</td>
</tr>
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<td>11</td>
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<td>27.5</td>
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<td>Mean</td>
<td>21.82</td>
<td>227.3</td>
<td>21.38</td>
<td>223.8</td>
</tr>
<tr>
<td>SEM</td>
<td>± 0.89</td>
<td>± 4.9</td>
<td>± 0.91</td>
<td>± 3.9</td>
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Table 3 - Biological variation of erythrocyte electrolyte concentration

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>First assay mmol/kg dry wt red cells</th>
<th>Second assay mmol/kg dry wt red cells</th>
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<td>4</td>
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<td>6</td>
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<td>233</td>
</tr>
<tr>
<td>23</td>
<td>22.9</td>
<td>206</td>
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</table>

Mean 22.26 228.01 21.60 232
SEM 0.82 4.0 0.72 3.9
**Biological variation of erythrocyte electrolyte concentration**

Blood samples were obtained on two occasions from twenty-three subjects and assayed for erythrocyte sodium and potassium concentration (Table 3). The interassay coefficient of variation calculated as described was 12.5% for intracellular sodium and 6.2% for intracellular potassium. The pooled mean for intracellular sodium was 21.9 mmol/kg dry weight with the pooled standard deviation of the pairs of ±2.75 and intracellular potassium 230 mmol/kg dry weight with a pooled standard deviation of ±14.2.

**ERYTHROCYTE SODIUM EFFLUX**

**Introduction**

Sodium efflux rate constant traditionally has been measured using radioactive sodium loaded washed cells incubated in physiological buffer (Sachs and Welt 1967). The rate of appearance of radioactivity in the medium being used to calculate the rate constant with a calculation devised by Hoffman (1966).
The basic calculation assumes that sodium extrusion is a first order process. The efflux rate constant is estimated in the absence and presence of ouabain (which inhibits the active sodium-potassium ATPase pump, Schatzman 1953). These are called the total and the ouabain resistant (residual) efflux rate constants respectively. The ouabain sensitive (active) sodium efflux rate constant is obtained by subtraction. Since the efflux rate constant is the proportion of intracellular sodium extruded per hour, sodium efflux can be calculated by multiplying the rate constant by the intracellular sodium concentration. A modification of the above method described by Cumberbatch and Morgan (1978) has been employed in these studies. The major change was the substitution of the subjects' own fresh plasma for the incubation medium. Haemolysis was prevented but more importantly the cells were always bathed in their own plasma which may contain stimulatory substances or any putative circulating sodium transport inhibitor (de Wardener and MacGregor 1980).

**Method:**

10 ml of fresh heparinised blood was centrifuged at 2000 g for 10 minutes and the plasma stored in a sealed tube at 4°C. A one ml aliquot of fresh heparinised blood was mixed with 1 ml of buffer containing 5 uCi of $^{22}$Na and placed in a shaking incubator at 37°C for 3 hours.
After the erythrocytes had been incubated for 3 hours, the suspension was divided into two tubes. 20 ml of cold (4°C) magnesium chloride (99 mmol/l) was added to each tube, mixed and then centrifuged at 13 000 g at 4°C in a Sorvall (R-C-SB) refrigerated superspeed centrifuge. The supernatant was removed and cells washed twice more with magnesium chloride and then finally with incubation buffer. Then the erythrocyte yield was combined and momentarily agitated with a vortex mixer to produce a homogenous suspension of Na²² loaded cells.

The pellet was divided into two tubes each containing 3.5 ml of the subjects own plasma (previously stored at 4°C in sealed tubes) to give a final haemocrit of approximately 6%. In addition, 50ul of saline was added to one tube and the same volume of saline containing ouabain (10 mmol/L) to the other. Immediately after mixing, a 200ul sample was removed into LP3 (Luckham, Ltd.) from each suspension before incubation in a shaking water bath at 37°C.

Thereafter, 400ul aliquots were transferred to microcentrifuge tubes at 15 minute intervals over a 75 minute incubation period. Each sample was centrifuged at 1500 g for 15 seconds in an Eppendorf micro centrifuge and the sodium²² content of 200ul of plasma (supernatant)
measured in a Packard gamma counter. At the end of the incubation period a 200ul aliquot of the incubation mixture was transferred into a LP3 tube. The sample of the suspension taken before and at the end of the incubation period was used to determine the total sodium$^{22}$ content of the erythrocyte suspension.

The efflux rate constants were calculated using the equation devised by Hoffman (Sachs and Welt 1967).

\[
- \log_e \frac{1-N_t}{N_0} = ERC \cdot t
\]

Where:

\(N_t\) = counts of sodium$^{22}$ present per unit volume of supernatant at time \(t\).

\(N_0\) = counts of sodium$^{22}$ present per unit volume of suspension of cells in plasma and was the mean of the value obtained at beginning and end of the incubation.

\(t\) = time in hours.

The slope of the linear regression between \(\log_e (1-N_t/N_0)\) and \(t\) was calculated by the least squares method and taken as the best estimate of the sodium efflux rate constant (ERC h$^{-1}$).
Active (glycoside sensitive) sodium efflux rate constant was obtained by subtracting the rate constant measured in the presence of ouabain from that obtained in the absence of ouabain. The rate of sodium efflux (mmol of sodium/h) was calculated by multiplying the ERC by the intracellular sodium concentration.

**Intra-assay variation of erythrocyte sodium efflux rate constant**

This was performed on the same blood samples used for determining the erythrocyte cation content. Efflux rate constant was measured on the two samples from the same subject and the coefficient of variation calculated as previously described.

**Results**

Individual values and means ± SEM are shown in Table 4. The pooled mean for the pairs ± the pooled standard deviation was 0.331±0.004 (Total), 0.134±0.021 (Ouabain resistant), 0.196±0.045 (ouabain sensitive). The intra-assay coefficient of variation of the paired samples was 11.3%, 15.4% and 23.1% respectively.
Table 4 - Intra-assay variation of erythrocyte sodium efflux rate constant (h⁻¹)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total</th>
<th>Ouabain</th>
<th>Ouabain</th>
<th>Total</th>
<th>Ouabain</th>
<th>Ouabain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Resistant</td>
<td>Sensitive</td>
<td></td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>1</td>
<td>0.330</td>
<td>0.132</td>
<td>0.198</td>
<td>0.30</td>
<td>0.114</td>
<td>0.186</td>
</tr>
<tr>
<td>2</td>
<td>0.360</td>
<td>0.226</td>
<td>0.132</td>
<td>0.378</td>
<td>0.15</td>
<td>0.228</td>
</tr>
<tr>
<td>3</td>
<td>0.330</td>
<td>0.102</td>
<td>0.228</td>
<td>0.252</td>
<td>0.126</td>
<td>0.126</td>
</tr>
<tr>
<td>4</td>
<td>0.312</td>
<td>0.132</td>
<td>0.180</td>
<td>0.306</td>
<td>0.126</td>
<td>0.180</td>
</tr>
<tr>
<td>5</td>
<td>0.372</td>
<td>0.138</td>
<td>0.234</td>
<td>0.30</td>
<td>0.138</td>
<td>0.162</td>
</tr>
<tr>
<td>6</td>
<td>0.228</td>
<td>0.126</td>
<td>0.102</td>
<td>0.222</td>
<td>0.078</td>
<td>0.144</td>
</tr>
<tr>
<td>7</td>
<td>0.348</td>
<td>0.114</td>
<td>0.234</td>
<td>0.288</td>
<td>0.120</td>
<td>0.168</td>
</tr>
<tr>
<td>8</td>
<td>0.282</td>
<td>0.132</td>
<td>0.150</td>
<td>0.390</td>
<td>0.150</td>
<td>0.240</td>
</tr>
<tr>
<td>9</td>
<td>0.504</td>
<td>0.162</td>
<td>0.342</td>
<td>0.534</td>
<td>0.150</td>
<td>0.384</td>
</tr>
<tr>
<td>10</td>
<td>0.414</td>
<td>0.174</td>
<td>0.240</td>
<td>0.408</td>
<td>0.162</td>
<td>0.246</td>
</tr>
<tr>
<td>11</td>
<td>0.216</td>
<td>0.102</td>
<td>0.114</td>
<td>0.204</td>
<td>0.102</td>
<td>0.102</td>
</tr>
<tr>
<td>Mean</td>
<td>0.336</td>
<td>0.140</td>
<td>0.196</td>
<td>0.326</td>
<td>0.129</td>
<td>0.197</td>
</tr>
<tr>
<td>SEM</td>
<td>±0.025</td>
<td>±0.011</td>
<td>±0.021</td>
<td>±0.029</td>
<td>±0.008</td>
<td>±0.023</td>
</tr>
</tbody>
</table>
Table 5 - Biological variation of erythrocyte sodium efflux rate constants (h⁻¹)

<table>
<thead>
<tr>
<th>Subject</th>
<th>First Assay</th>
<th></th>
<th></th>
<th>Second Assay</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Ouabain</td>
<td>Ouabain</td>
<td>Total</td>
<td>Ouabain</td>
<td>Ouabain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resistant</td>
<td>Sensitive</td>
<td></td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>1</td>
<td>0.252</td>
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<td>0.174</td>
<td>0.558</td>
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<td>0.414</td>
</tr>
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<td>0.132</td>
<td>0.516</td>
<td>0.162</td>
<td>0.354</td>
</tr>
<tr>
<td>3</td>
<td>0.300</td>
<td>0.120</td>
<td>0.180</td>
<td>0.402</td>
<td>0.108</td>
<td>0.294</td>
</tr>
<tr>
<td>4</td>
<td>0.108</td>
<td>0.090</td>
<td>0.018</td>
<td>0.300</td>
<td>0.138</td>
<td>0.162</td>
</tr>
<tr>
<td>5</td>
<td>0.252</td>
<td>0.090</td>
<td>0.162</td>
<td>0.294</td>
<td>0.108</td>
<td>0.186</td>
</tr>
<tr>
<td>6</td>
<td>0.192</td>
<td>0.108</td>
<td>0.084</td>
<td>0.438</td>
<td>0.150</td>
<td>0.288</td>
</tr>
<tr>
<td>7</td>
<td>0.258</td>
<td>0.090</td>
<td>0.168</td>
<td>0.384</td>
<td>0.108</td>
<td>0.276</td>
</tr>
<tr>
<td>8</td>
<td>0.180</td>
<td>0.066</td>
<td>0.114</td>
<td>0.318</td>
<td>0.204</td>
<td>0.114</td>
</tr>
<tr>
<td>9</td>
<td>0.240</td>
<td>0.072</td>
<td>0.168</td>
<td>0.288</td>
<td>0.102</td>
<td>0.186</td>
</tr>
<tr>
<td>10</td>
<td>0.180</td>
<td>0.060</td>
<td>0.120</td>
<td>0.270</td>
<td>0.084</td>
<td>0.186</td>
</tr>
<tr>
<td>11</td>
<td>0.156</td>
<td>0.090</td>
<td>0.066</td>
<td>0.252</td>
<td>0.078</td>
<td>0.174</td>
</tr>
<tr>
<td>12</td>
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<td>0.372</td>
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<tr>
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<td>0.324</td>
<td>0.114</td>
<td>0.210</td>
</tr>
<tr>
<td>14</td>
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<td>0.084</td>
<td>0.360</td>
<td>0.114</td>
<td>0.246</td>
</tr>
<tr>
<td>15</td>
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<td>0.182</td>
<td>0.342</td>
<td>0.096</td>
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<tr>
<td>16</td>
<td>0.156</td>
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<td>0.078</td>
<td>0.210</td>
<td>0.096</td>
<td>0.114</td>
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<tr>
<td>17</td>
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<td>0.054</td>
<td>0.276</td>
<td>0.078</td>
<td>0.198</td>
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<tr>
<td>18</td>
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<td>0.138</td>
<td>0.234</td>
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<td>0.150</td>
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<tr>
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<td>0.252</td>
<td>0.360</td>
<td>0.126</td>
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<td>20</td>
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<td>0.042</td>
<td>0.222</td>
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<td>0.132</td>
</tr>
<tr>
<td>21</td>
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<td>0.078</td>
<td>0.171</td>
<td>0.300</td>
<td>0.090</td>
<td>0.210</td>
</tr>
<tr>
<td>22</td>
<td>0.240</td>
<td>0.090</td>
<td>0.150</td>
<td>0.300</td>
<td>0.114</td>
<td>0.186</td>
</tr>
<tr>
<td>23</td>
<td>0.156</td>
<td>0.090</td>
<td>0.066</td>
<td>0.162</td>
<td>0.090</td>
<td>0.072</td>
</tr>
<tr>
<td>Mean</td>
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<td>0.097</td>
<td>0.138</td>
<td>0.321</td>
<td>0.112</td>
<td>0.209</td>
</tr>
<tr>
<td>SEM</td>
<td>± 0.178</td>
<td>± 0.006</td>
<td>± 0.016</td>
<td>± 0.019</td>
<td>± 0.006</td>
<td>± 0.017</td>
</tr>
</tbody>
</table>
Biological variation of erythrocyte sodium efflux:

Blood samples from twenty-three (23) normotensive subjects were taken for sodium efflux rate constant determination on two separate occasions to measure the biological variation of the assay.

Results:

Individual values and means ± SEM are shown in Table 5. The pooled mean values ± pooled standard deviation of the pairs was 0.278±0.80 (Total), 0.105±0.030 (Ouabain resistant), 0.174±0.067 (Ouabain sensitive). Coefficient of biological variation was 28.7%, 38.8% and 28.9% respectively.

PLASMA RENIN ACTIVITY (PRA)

Ten mls of blood was collected into pre-chilled vacutainer tubes containing 50 mg potassium ethylenediaminetetraacetic acid (EDTA) and the plasma separated at 4°C and stored until assayed. PRA was measured by radioimmunoassay of generated angiotensin I as previously described by Swales and Thurston (1977).
ERYTHROCYTE MEMBRANE LIPID ANALYSIS

The technique used was a modification of that described by Sanders, Ellis and Dickerson (1978). A 10 ml sample of venous blood was drawn into a lithium heparin vacutainer and placed on ice for 90 minutes. The blood was then centrifuged at 3000 rpm for 15 minutes at 4°C and the plasma removed. 4 ml of the sedimented erythrocytes were added to 5 ml of saline ethylenediaminetetraacetic acid (EDTA) solution in a sterilin tube placed on ice. A further 15 ml of saline/EDTA was added and the suspension centrifuged at 2800 rpm, for 12 minutes at 4°C. The supernatant was removed and 20 ml of saline/EDTA added before mixing and centrifuging again. A further wash was performed and the erythrocyte pellets separated.

Extraction

1 ml of washed cells was taken in each of two "Jumbo" glass centrifuge tubes and equal volume of distilled water added. The tubes were kept on ice for 15 minutes while cell lysis occurred. After this, 11 ml of propanol/butylated hydroxytoluene (BHT) solution was added to each tube, the tubes were mixed and kept on ice for an hour, 7 ml of chloroform was added to each tube, the contents mixed and left on ice for a further hour prior to centrifugation at
1500 rpm for ten minutes at 4°C. The supernatant was removed and stored in a glass universal container at 20°C.

**Methylation**

The contents stored in the glass universal containers were thawed, placed in a water bath at 55°C and taken to dryness under a stream of oxygen free nitrogen. 0.3 ml hexane and 0.1 ml sodium methoxide solution were pipetted on to the residue and the tube placed on a spiramix for 5 minutes. After complete mixing, 2.5 ml of hexane and a small amount (approximately 0.5 g) of anhydrous calcium chloride was added to each tube. After one hour, the contents of each tube were filtered through a Whatman no.1 filter disk soaked in hexane. The residue was washed once with 2.5 ml hexane and filtered. The pooled filtrates were separated to dryness under nitrogen as before and taken up into 0.2 ml hexane for analysis by gas liquid chromatography.

**Chromatography**

0.5 to 1.0 ul of each sample was injected into a Perkin Elmer Model F17 gas liquid chromatograph fitted with a 2 m x 0.5" glass column containing 15% diethylene glycol succinate (DEGS) on Chromosorb W (100-200 mesh) and a flame ionisation
Fig 2: A specimen chromatogram of the fatty acid methyl ester standards (FAMES, 14.0 - Myristic; 16.0 - Palmitic; 18.0 - Stearic; 18.1 - oleic; 18.2, linoleic; 18.3 - Linolenic; 18.3 - Eleostearic; 21.0 - Meneicosanoic; 20.4 - Arachidonic acid). The numbers above the peaks denote the retention times in minutes.
Fig 3: A typical chromatogram of the fatty acid methyl esters of human erythrocytes. The values above the peaks denote the retention times in minutes. The fatty acids isolated are 16.0 Palmitic; 18.0 Stearic; 18.1 Oleic; 18.2 Linoleic; 18.3 Linolenic; 20.4 Arachidonic acid. All the peaks before 4.67 minutes and the peaks at 6.29 and 7.47 minutes are due to components of the reaction mixture and appear on chromatograms containing no fatty acid methyl esters.
Table 6

Shimadzu computer integrator analysis of the erythrocyte fatty acid chromatogram shown in Fig 6.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Retention time (minutes)</th>
<th>Peak Height</th>
<th>% in Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.0 Palmitic</td>
<td>5.47</td>
<td>11960</td>
<td>40.6 %</td>
</tr>
<tr>
<td>18.0 Stearic</td>
<td>9.79</td>
<td>6341</td>
<td>21.6 %</td>
</tr>
<tr>
<td>18.1 Oleic</td>
<td>11.09</td>
<td>5232</td>
<td>17.9 %</td>
</tr>
<tr>
<td>18.2 Linoleic</td>
<td>13.69</td>
<td>3634</td>
<td>12.4 %</td>
</tr>
<tr>
<td>20.4 Arachidonic</td>
<td>31.96</td>
<td>2219</td>
<td>7.5 %</td>
</tr>
</tbody>
</table>
detector. The oven was run isothermically at a temperature of 180°C, the injector/detector temperature was 250°C and nitrogen was the carrier gas at a flow rate of 55 ml/minute.

Fatty acid methyl esters were identified by comparing the retention times with those of standards (Fig 2). Peak heights were measured with the aid of a Shimadzu computing integrator and the individual fatty acids expressed as a percentage relative to all the fatty acids methyl esters chromatographed (see Fig 3 and Table 6).

**Statistical analysis**

All the data collected for this thesis was stored on hard disk in a PDP-11-23 digital computer. Analysis was performed by Student's "t" test, Man Whitney Test or Analysis of variance as appropriate using a Minitab (1982) statistical package from Pennsylvania State University.
CHAPTER 3

ERYTHROCYTE SODIUM TRANSPORT IN NORMOTENSIVE CONTROLS,
PATIENTS WITH ESSENTIAL HYPERTENSION AND THEIR
NORMOTENSIVE OFFSPRING.
Unselected normotensive subjects

A preliminary study to determine the influence of sex on erythrocyte sodium transport was carried out using a group of fifty-eight subjects (thirty males and twenty-eight females). All were white, sixteen of the females and fourteen of the males had no family history of hypertension but the rest had at least one first degree relative with hypertension. None of the subjects were receiving regular medication (including the contraceptive pill since this is known to increase sodium-lithium cotransport (Beucklman, Erdmann 1984) and on the day of study continued their normal activities and diet.

Each subject had their height, weight and lying and standing blood pressure measured before a blood sample was obtained for plasma renin, erythrocyte cation content and sodium transport. After this, each subject collected twenty-four hour urine samples to determine sodium and potassium excretion.
Table 7 - Shows the age, weight, height, blood pressure, urinary electrolyte excretion and plasma renin activity (mean ±SEM) in 58 normotensive subjects.

<table>
<thead>
<tr>
<th></th>
<th>Urinary Sodium</th>
<th>Urinary Potassium</th>
<th>PRA (mmol/24h)</th>
<th>PRA (ngAI/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Age (yrs)</td>
<td>Weight (Kg)</td>
<td>Height (M)</td>
<td>BP in mmHg</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>25±1.1</td>
<td>56.7±1.3</td>
<td>1.62±0.01</td>
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<tr>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Age (yrs)</td>
<td>Weight (Kg)</td>
<td>Height (M)</td>
<td>BP in mmHg</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>25±1.0</td>
<td>76±1.7</td>
<td>1.79±0.00*</td>
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<td></td>
<td></td>
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<td></td>
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</table>

*(P < 0.01)*
Table 8 - Sodium efflux rate constants and intracellular electrolyte content in males and females

<table>
<thead>
<tr>
<th></th>
<th>Sodium efflux rate constant (h)^{-1}</th>
<th>Active sodium efflux (mmol/kg dry wt/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ouabain Resist. Sens.</td>
<td></td>
</tr>
<tr>
<td>Intracellular</td>
<td>Sodium efflux rate constant</td>
<td></td>
</tr>
<tr>
<td>electrolytes</td>
<td>(mmol/Kg dry wt.)</td>
<td></td>
</tr>
<tr>
<td>(Na)\textsubscript{i}</td>
<td>(K)\textsubscript{i}</td>
<td>Total</td>
</tr>
<tr>
<td>(mmol/Kg dry wt.)</td>
<td>(mmol/kg dry wt/h)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F. 19.5±0.07 237±2.9 0.326±0.025 0.124±0.008 0.202±0.02</td>
<td>3.8±0.35</td>
</tr>
<tr>
<td></td>
<td>M 21.7±0.84 226±4.7 0.360±0.027 0.130±0.008 0.230±0.022</td>
<td>4.8±0.45</td>
</tr>
</tbody>
</table>

*(p < 0.05)*
Fig 4: Erythrocyte sodium content (mmol/kg dry weight) of age matched male and female normotensive subjects.
Fig 5: Sodium efflux rate constants (h\(^{-1}\)) of age matched male and female normotensive subjects.
The physical characteristics of this group are shown in Table 7. Although the males and females were well matched for age, the males were significantly taller, heavier with a greater supine blood pressure and urine sodium excretion. Despite these differences, plasma renin activity was similar in the males and females.

Erythrocyte sodium content was higher in the males ($p<0.05$) but although sodium efflux rate was also higher, this did not achieve statistical significance (Table 8). Figures 4 and 5 show the erythrocyte sodium content and the sodium efflux rate constants for the males and females. In the group as a whole there was no correlation between age and erythrocyte sodium content or sodium efflux rate constant or blood pressure.

Although similar numbers of the males and females in this study had a family history of hypertension, this may have influenced the sex differences observed. However, when analysis was performed taking account of the family history, the same sex trends were observed. Nevertheless, it was decided to use only normotensive subjects without a family history of hypertension as controls in future experiments.
Normotensive subjects without a family history of hypertension

Thirty seven (37) subjects, twenty (20) males and seventeen (17) females were studied. All were white, none had a family history of hypertension, none of the subjects were receiving regular medication (including the contraceptive pill) and were on normal diet with usual daily activities. The physical characteristics of this group are shown in Table 9. They were well matched for age and blood pressure, but males were significantly heavier \( (p < 0.001) \) and taller \( (p = < 0.01) \). Twenty-four hour (24h) urinary excretion of sodium \( (p < 0.01) \) and potassium \( (p < 0.001) \) were significantly higher in males than females.

Intracellular sodium was higher in males compared to females \( (21.22 \pm 0.86 \text{ vs } 19.34 \pm 0.82) \), but the difference just failed to achieve significance \( (p > 0.05) \). The efflux rate constants were not significantly different (Table 10). The mean values for all basic characteristics, intracellular electrolytes and efflux rate constants, of the whole group of subjects are shown in Table 11 & 12, Figs 6 & 7.
Table 9 - The physical characteristics of the 37 normotensive control subjects without a family history of hypertension

<table>
<thead>
<tr>
<th>Age (Kg)</th>
<th>Weight (M)</th>
<th>Height (M)</th>
<th>Blood Pressure (mmHg)</th>
<th>Urinary Excretion (mmol/24 h)</th>
<th>PRA (ngAI/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.5±1.6</td>
<td>57.7±1.7</td>
<td>1.64±0.02</td>
<td>123.8±3</td>
<td>114.6±2.4</td>
<td>126.4±2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>71.4±2.8</td>
<td>79±1.8</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26.3±1.6</td>
<td>75.4±2.3</td>
<td>1.84±0.05</td>
<td>130±3.9</td>
<td>121±4</td>
<td>167.2±9.2</td>
</tr>
</tbody>
</table>

** p < 0.001
* p < 0.01
Table 10 - Sodium efflux rate constant, intracellular electrolyte content (mmol/kg dry weight) in 20 males and 17 females

<table>
<thead>
<tr>
<th></th>
<th>Mean Efflux Rate Constant (h^{-1})</th>
<th>Intracellular Electrolyte Content</th>
<th>Active Sodium Efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total (mmol/kg dry weight)</td>
<td>(mmol/kg dry wt/h)</td>
</tr>
<tr>
<td>Femaless</td>
<td></td>
<td>Ouabain (Resistant) [Na]_i [K]_i</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.325 ( \pm ) 0.028</td>
<td>0.119 ( \pm ) 0.010</td>
<td>19.30 ( \pm ) 0.82</td>
</tr>
</tbody>
</table>

| Males    |                                    | 0.122 \( \pm \) 0.009             | 21.22 \( \pm \) 0.86 | 230 \( \pm \) 6.5 | 4.9 \( \pm \) 0.56 |

|          |                                    | 0.346 \( \pm \) 0.03              | 0.227 \( \pm \) 0.024 |                      |                      |
Table 11 - Shows the age, weight, height, blood pressure, urinary electrolyte excretion and plasma renin activity in 37 normotensive subjects

<table>
<thead>
<tr>
<th>No.</th>
<th>Age  (yrs)</th>
<th>Weight (kg)</th>
<th>Height (M)</th>
<th>BP in mmHg (Supine, Standing)</th>
<th>UNa</th>
<th>UK</th>
<th>PRA (ngAI/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>25.95±1.1</td>
<td>67.2±2.1</td>
<td>1.74±0.03</td>
<td>127.2±2.5, 118.0±2.4</td>
<td>74±3</td>
<td>4.92±0.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>68.1±2.2, 78.27±1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 12 - Sodium efflux rate constant, intracellular electrolyte content in 37 normotensive subjects

<table>
<thead>
<tr>
<th>Mean Efflux Rate Constant (h⁻¹)</th>
<th>Intracellular Electrolyte Content (mmol/Kg dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>[Na]i  [K]i</td>
</tr>
<tr>
<td>Ouabain Resistant</td>
<td>0.121±0.0065  0.217±0.017</td>
</tr>
<tr>
<td>Ouabain Sensitive</td>
<td>0.337±0.02  20.34±0.61</td>
</tr>
<tr>
<td></td>
<td>233.9±4.0</td>
</tr>
</tbody>
</table>
Fig 6: Erythrocyte sodium content (mmol/kg/dry weight) of age matched male and female normotensive subjects with no family history of hypertension.
Fig 7: Sodium efflux rate constants (h⁻¹) of age matched male and female subjects with no family history of hypertension.
Hypertensive Patients

Seventeen (7 males and 10 females) untreated essential hypertensive patients, aged 19–65 years attending the hypertension clinic of the Leicester Royal Infirmary were studied. None had evidence of a cause of their hypertension or had previously been given antihypertensive treatment. These were compared to seventeen (7 males and 10 females) normotensive control subjects aged 19–45 years who had no history of hypertension or other disease. All of the subjects were white Caucasians, taking an unrestricted diet and none had evidence of renal or cardiac disease or were taking regular medication (including the oral contraceptive pill).

Blood pressure was recorded in the supine and standing positions using a Hawksley Random zero sphygmonanometer. All subjects were screened with a blood count and routine biochemistry for serum electrolytes, urea, creatinine and liver function, but in addition an electrocardiogram and chest radiography were performed on the hypertensive patients.
Results

Clinical details of the normotensive control subjects and the hypertensive patients are shown in Table 13. In addition to the difference in blood pressure hypertensive subjects were significantly heavier than the controls but were not well matched for age with normotensive subjects. There was no significant difference in height or plasma renin activity between the two groups.

Intracellular sodium was 20.5±0.8 mmol/kg dry weight of cells in the controls and 22.6±0.9 mmol/kg dry weight in the essential hypertensives (p = 0.05). Individual values for erythrocyte sodium content for the two groups are shown in Figure 8. There was no significant difference in intracellular potassium which was 229±4 in the controls and 231±4 mmol/kg dry weight in the hypertensives (p > 0.05). Erythrocyte total sodium efflux rate constant and ouabain resistant component were significantly higher in the hypertensive subjects (p < 0.05, See Table 14 and Fig. 9). However, there was no significant difference in the ouabain sensitive (active pump) sodium efflux of 0.213±0.02) and (0.207±0.03) respectively). The active sodium efflux rate (ouabain sensitive efflux rate constant multiplied by the intracellular sodium) was not significantly different in the hypertensives compared to the normal controls (Table 14).
Fig 8: Erythrocyte sodium content (mmol/kg dry weight) of normotensive controls and patients with essential hypertension.
Table 13 - Showing the clinical characteristics of the normotensive controls and patients with essential hypertension

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Height (M)</th>
<th>Blood Pressure (mm Hg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Supine</td>
<td>Standing</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>F:7</td>
<td>30±1.9</td>
<td>72.5±3.4</td>
<td>1.74±0.02</td>
<td>127±4</td>
<td>118±3</td>
</tr>
<tr>
<td></td>
<td>M:10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>70±4</td>
<td>78±3</td>
</tr>
</tbody>
</table>

Hypertensives

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Height (M)</th>
<th>Blood Pressure (mm Hg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Supine</td>
<td>Standing</td>
</tr>
<tr>
<td>17</td>
<td>F:7</td>
<td>48±1.9</td>
<td>74.8±3.8</td>
<td>1.69±0.03</td>
<td>174±6 *</td>
<td>167±7 *</td>
</tr>
<tr>
<td></td>
<td>M:10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>107±4 *</td>
<td>110±3 *</td>
</tr>
</tbody>
</table>

* p < 0.001
### Table 14 - Mean sodium efflux rate constants, intracellular electrolyte content and efflux rates in normotensive controls and hypertensive subjects.

<table>
<thead>
<tr>
<th></th>
<th>Sodium Efflux Rate Constant (h⁻¹)</th>
<th>Intracellular Electrolyte (mmol/Kg dry weight)</th>
<th>Sodium Efflux Rate (mmol/kg dry wt/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ouabain</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Normotensives</strong></td>
<td>0.313 ± 0.032</td>
<td>0.111 ± 0.008</td>
<td>0.207 ± 0.028</td>
</tr>
<tr>
<td></td>
<td>0.111 ± 0.008</td>
<td>20.5 ± 0.8</td>
<td>229 ± 4</td>
</tr>
<tr>
<td></td>
<td>0.207 ± 0.028</td>
<td></td>
<td>4.2 ± 0.44</td>
</tr>
<tr>
<td><strong>Hypertensives</strong></td>
<td>0.354 ± 0.027</td>
<td>0.146 ± 0.014</td>
<td>0.213 ± 0.023</td>
</tr>
<tr>
<td></td>
<td>0.146 ± 0.014</td>
<td>22.6 ± 0.9</td>
<td>231 ± 4</td>
</tr>
<tr>
<td></td>
<td>0.213 ± 0.023</td>
<td></td>
<td>4.64 ± 0.59</td>
</tr>
</tbody>
</table>

* p = 0.05  ** p < 0.05
Fig 9: Sodium efflux rate constants (h⁻¹) of normotensive controls (N) and patients with essential hypertension (H).
Normotensive Offspring of Hypertensive Subjects

These were defined as subjects who had one or more first degree relatives known to be on treatment for hypertension. Initial classification was by interview, but where there was doubt, the blood pressure of the parents was measured in the Department of Medicine or by their own general practitioner. Twenty-one normotensive relatives (10 males and 11 females) were compared to twenty-one control subjects (with no family history of hypertension) matched for age, weight and sex. There was no significant difference between the two groups regarding their physical characteristics (see Table 15). Erythrocyte intracellular sodium content was higher in the relatives, but this difference just failed to achieve statistical significance (Table 16, Fig 10). There was no significant difference in any component of the sodium efflux rate constant or active sodium efflux rate between the controls or the relatives of hypertensive patients (Table 16, Fig 11).

Discussion

The study performed on a large group of normotensive subjects confirms previous reports by Naylor (1970) and Weissberg, West and Woods (1983) that erythrocyte sodium content is significantly higher in males than females. Furthermore, since sodium efflux rate constant was the same
Table 15 - Physical characteristics of normotensive offspring of hypertensive patients and matched controls

<table>
<thead>
<tr>
<th>Number</th>
<th>Age</th>
<th>Weight</th>
<th>Height</th>
<th>Blood Pressure</th>
<th>UNa</th>
<th>UK</th>
<th>PRA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(yrs)</td>
<td>(Kg)</td>
<td>(M)</td>
<td>Supine</td>
<td>Standing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>M:10</td>
<td>24±0.7</td>
<td>66±2.4</td>
<td>1.70±0.02</td>
<td>128±3.5</td>
<td>115±2.7</td>
<td>142±7.8</td>
</tr>
<tr>
<td></td>
<td>F:11</td>
<td></td>
<td></td>
<td></td>
<td>68±3.2</td>
<td>77±2.1</td>
<td></td>
</tr>
<tr>
<td>Relatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>M:10</td>
<td>24±0.8</td>
<td>65±2.4</td>
<td>1.69±0.02</td>
<td>129±2.2</td>
<td>120±2.3</td>
<td>140±12.3</td>
</tr>
<tr>
<td></td>
<td>F:11</td>
<td></td>
<td></td>
<td></td>
<td>65±2.7</td>
<td>73±1.8</td>
<td></td>
</tr>
</tbody>
</table>
Table 16 - Erythrocyte intracellular electrolyte content and efflux rate constants of normotensive controls and relatives of hypertensive patients

<table>
<thead>
<tr>
<th></th>
<th>Sodium Efflux Rate Constant (h⁻¹)</th>
<th>Intracellular Electrolytes</th>
<th>Active Sodium Efflux Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ouabain</td>
<td>[Na]ᵢ</td>
<td>[K]ᵢ</td>
</tr>
<tr>
<td>Total Controls</td>
<td>Resistant</td>
<td>0.132±0.009</td>
<td>0.231±0.024</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
<td>0.363±0.29</td>
<td>0.132±0.009</td>
</tr>
<tr>
<td></td>
<td>Ouabain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relatives</td>
<td>Ouabain</td>
<td>0.136±0.009</td>
<td>0.211±0.028</td>
</tr>
<tr>
<td></td>
<td>Ouabain</td>
<td>0.347±0.035</td>
<td>0.136±0.009</td>
</tr>
</tbody>
</table>
Fig 10: Erythrocyte sodium content (mmol/kg dry weight) of normotensive controls and normotensive relatives of hypertensive patients.
Fig 11: Sodium efflux rate constants ($h^{-1}$) of normotensive controls (C) and the normotensive relatives of hypertensive patients (R).
it must be concluded that passive sodium permeability also
is greater in males. Only a few studies using the white
cell are available for comparison, but Heagerty (1987)
failed to observe a difference in intracellular sodium
between males and females. Moreover, in sharp contrast the
majority of studies with erythrocytes where sodium content
is higher in older subjects he found a significant negative
correlation between leucocyte sodium and age. Only
Caucasian subjects were used in my studies and males and
females were well age matched. However, these results again
emphasise the considerable influence of factors such as
race, sex and age on membrane sodium handling and that
studies in essential hypertension must employ controls
carefully matched to the patients.

The patients with untreated essential hypertension like
others previously reported (Losse, Wehmeyer and Wessels
1960; Clegg, Morgan and Davidson 1982) had a significantly
elevated erythrocyte sodium. They also showed a significant
increase in both sodium efflux rate constant and absolute
sodium efflux rate confirming that essential hypertension is
associated with elevated erythrocyte sodium pumping
(Fitzgibbon, Morgan and Myers 1980; Woods, Beevers and West
1981a; Boon, Harper, Aronson et al 1984). In the
normotensive subjects with a family history of hypertension,
intracellular sodium showed a small increase but sodium
pumping was no different from the control subjects. Gudmundsson, (1984a) also reported a significant elevation of intra-erythrocyte sodium in young men with a positive family history of hypertension but found sodium influx was unchanged and the sodium efflux rate constant reduced. Misclassification of the subjects could explain the failure to demonstrate significant differences in the present study since this would tend to dilute out differences between normotensives with and without a positive family history of hypertension. Similarly, a study of leucocytes from these subjects has shown that intracellular sodium and total unidirectional sodium efflux rate for sodium were higher but these did not achieve statistical significance (Heagerty, Milner, Bing et al 1982; Milner, Heagerty, Bing et al 1984). However, total and ouabain sodium efflux rate constants were significantly depressed in leucocytes of the normotensive offspring of hypertensive patients.

The present experiments have demonstrated significant small changes in membrane sodium handling in erythrocytes of hypertensive patients and similar changes in the normotensive offspring which did not reach statistical significance. However, the hypertensives were significantly older than the controls but in my studies similar age differences in normotensive subjects were not associated with significant changes in intracellular sodium.
Nevertheless, taking previous and the present observations together suggests that intra-erythrocyte sodium is raised in essential hypertension but this was not associated with a fall in sodium pump activity, implying that passive sodium influx is elevated. Thus, the baseline studies do not lend support to the hypothesis of de Wardener and MacGregor (1980). This hypothesis proposes that the development of essential hypertension in humans is due to an inherited defect in the ability of the kidney to excrete sodium. The excess salt in the Western diet results in a tendency towards sodium retention and blood volume expansion. Consequently, the level of a circulating sodium transport inhibitor would rise to correct volume expansion by enhancing renal sodium excretion. As a side effect sodium transport would be depressed in circulating blood cells and more importantly vascular smooth muscle. In vascular smooth muscle, the consequent rise in intracellular sodium would then depress the sodium:calcium exchange mechanism in the plasma membrane. The net result would be a rise in intracellular calcium and thus an increase in vascular reactivity and thereby hypertension. The elevation of sodium pump activity and the absence of any evidence to support blood volume expansion in essential hypertension contradicts this hypothesis. Furthermore, changes have been demonstrated in the normotensive offspring at a time when their blood pressure is normal. In rebuttal, it has been
argued that compensatory mechanisms are activated to keep the blood pressure from rising in young offspring of hypertensive patients and only in later life does the blood pressure rise. However, no such timelag was envisaged by the original hypothesis of Blaustein (1977) from which the theory of de Wardener and MacGregor (1980) was developed. A crucial test is whether volume expansion will inhibit sodium pumping in genetically susceptible normotensive subjects. Accordingly it was decided to study the effects of changes in sodium balance in two groups of subjects, normotensive offspring of essential hypertensives and controls and these experiments are described in Chapter 4.
CHAPTER 4

STUDIES OF THE EFFECTS OF CHANGING DIETARY SALT INTAKE ON BLOOD PRESSURE AND ERYTHROCYTE SODIUM TRANSPORT IN NORMOTENSIVE OFFSPRING OF HYPERTENSIVE PATIENTS AND MATCHED CONTROLS.
Introduction

The studies reported in Chapter 3 have shown that there are abnormalities of membrane sodium handling in essential hypertensive patients and their normotensive offspring. Consequently it is unlikely that these changes are directly involved in blood pressure elevation. However, the finding that the blood pressure fall induced by diuretics in hypertensive patients is associated with a return of the sodium pump activity and intracellular sodium to normal (Poston, Jones, Richardson et al 1981b; Araoye, Khatri, Yao et al 1978) does favour the hypothesis of de Wardener & MacGregor. On the other hand the administration of a thiazide diuretic for seven days to the normotensive first degree relatives of hypertensive patients also brought about an increase in leucocyte sodium-potassium ATPase activity to normal without a significant fall in blood pressure (Milner, Heagerty, Bing et al 1984). It is important therefore to investigate the effects of altering sodium balance by manipulation of dietary salt intake in subjects who are genetically at risk of developing hypertension.
Subjects

Thirteen (8 male, 5 female) healthy normotensive subjects, mean age 25 years (range 19-45 years) all with one or more first degree relatives known to have essential hypertension were studied. These subjects were compared with 13 sex and age-matched (range 19-33 years) normotensive controls with no family history of hypertension. All subjects were Caucasian and there were no significant differences in age, weight, plasma renin activity (PRA) or blood pressure between the groups (Table 17).

Study Protocol

The daily caloric intake of each subject was assessed by the Senior Dietician of Leicester Royal Infirmary. On entry into the study, weight, height and blood pressure were measured and each subject was asked to provide three 24 hour urine collections to ascertain the dietary sodium and potassium intake. A venous blood sample was obtained to measure plasma renin activity (PRA) and erythrocyte electrolyte content and sodium transport. Subjects were randomized to receive either a low or high sodium intake for 14 days. At the end of the dietary period each volunteer provided a further 24 hour urine collection and blood was
drawn for PRA, erythrocyte electrolyte content and sodium transport studies. There followed a two week washout period of their normal diets before the subjects completed the other arm of the dietary study with restudy after a further fourteen days. The low salt diet (40 mmol sodium/day), with an identical caloric value to their normal diet was provided by the diet kitchen at the Leicester Royal Infirmary. A high intake was achieved by supplementing the subjects 150 mmol/sodium, with 15 slow sodium tablets (150 mmol sodium) Ciba Labs, Horsham (UK), so as to double the salt intake. Urinary electrolyte estimations were performed using a Corning flame photometer with a lithium internal standard. Blood pressures were measured in the supine and standing positions with a Hawksley Random Zero Sphygmomanometer, three readings were taken in each position and mean value recorded.

Results

There were no significant differences in baseline clinical characteristics between the two groups on their normal diet (Table 17). All the subjects recruited to the study completed both arms of the protocol. Many subjects commented that the low salt diet was bland and unpleasant but the slow sodium tablets caused no ill-effects. Analysis of the data within each group was performed by Student's
Table 17 - Clinical characteristics of the normotensive offspring of hypertensive patients and control subjects on normal diet

<table>
<thead>
<tr>
<th></th>
<th>Age (yrs)</th>
<th>Weight (Kg)</th>
<th>BP (mm Hg)</th>
<th>UNa mmol/24h</th>
<th>UK</th>
<th>PRA ngAI/ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Supine</td>
<td>Standing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 M:8</td>
<td>27±2.0</td>
<td>71±3.9</td>
<td>129±3.4</td>
<td>118±3.6</td>
<td>147±14.5</td>
<td>78±3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72±4.5</td>
<td>77±3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F:5</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 M:8</td>
<td>24±1.2</td>
<td>69±3.3</td>
<td>127±2.9</td>
<td>121±3.2</td>
<td>137±17.2</td>
<td>65±6.8</td>
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<td></td>
<td></td>
<td></td>
<td>65±3.8</td>
<td>76±2.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 18 - Sodium efflux rate constant (h^{-1}), intracellular electrolyte content (mmol/kg dry weight) and active sodium efflux rate (mmol/kg dry wt/h) in normotensive offspring of hypertensive patients and controls on normal diet.

<table>
<thead>
<tr>
<th></th>
<th>Mean efflux rate constant (h^{-1})</th>
<th>Intracellular Electrolyte content (mmol/kg dry wt)</th>
<th>Active Sodium efflux rate (mmol/kg dry weight/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ouabain</td>
<td>(Na)i</td>
<td>(K)i</td>
</tr>
<tr>
<td>Total Controls</td>
<td>Total</td>
<td>0.284±0.038</td>
<td>0.105±0.011</td>
</tr>
<tr>
<td></td>
<td>Ouabain Resistant</td>
<td>0.105±0.011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ouabain Sensitive</td>
<td>0.178±0.034</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Na)i</td>
<td>22±0.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(K)i</td>
<td>233±3.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Active Sodium efflux rate (mmol/kg dry weight/h)</td>
<td>3.9±0.80</td>
<td></td>
</tr>
<tr>
<td>Relatives</td>
<td>Total</td>
<td>0.276±0.042</td>
<td>0.118±0.011</td>
</tr>
<tr>
<td></td>
<td>Ouabain Resistant</td>
<td>0.118±0.011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ouabain Sensitive</td>
<td>0.157±0.033</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Na)i</td>
<td>23±1.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(K)i</td>
<td>225±4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Active Sodium efflux rate (mmol/kg dry weight/h)</td>
<td>3.4±0.64</td>
<td></td>
</tr>
</tbody>
</table>
paired "t" test, comparing the data from the low and high salt periods only, these being the randomised dietary periods (Hills and Armitage 1979). The pattern of response in the two groups to the two salt intake regimes was compared by analysis of variance.

Mean body weight was significantly lower on the low salt compared to the high salt diet ($p < 0.001$), Table 19. Urinary sodium excretion and plasma renin activity changed appropriately in response to the dietary manipulation confirming good compliance (Tables 19 & 21).

**Blood Pressure**

There was no significant difference between the blood pressure of the two groups in either dietary period. In neither group was the supine blood pressure different on the low compared to the high salt diet (Tables 19 & 21). However, in the controls both standing systolic and diastolic blood pressure was significantly reduced on low salt diet in the controls ($p < 0.01$ - Table 19) but the fall in standing systolic blood pressure was not significant in relatives ($p > 0.05$ - Table 21). Taking both groups as a whole, there was a significant fall in standing blood pressure ($p < 0.01$ ANOVA), but the postural changes did not cause symptoms in any subject.
Table 19 - Body weight, blood pressure, daily urinary sodium and potassium excretion, plasma renin activity on low and high salt intakes in the control subjects.

<table>
<thead>
<tr>
<th>Dietary Period</th>
<th>Weight</th>
<th>BP (mm Hg)</th>
<th>UNa</th>
<th>UK</th>
<th>PRA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Supine</td>
<td>Standing</td>
<td>mmol/24 h</td>
<td>ng/Al/ml</td>
</tr>
<tr>
<td>Low Salt</td>
<td>69±3.6***</td>
<td>127±2.7</td>
<td>109±3.7**</td>
<td>36±3.7***</td>
<td>71±4.2</td>
</tr>
<tr>
<td></td>
<td>69±3.2</td>
<td>74±3.8**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High salt</td>
<td>70±3.8</td>
<td>127±2.8</td>
<td>121±4.0</td>
<td>268±15.9</td>
<td>78±6.8</td>
</tr>
<tr>
<td></td>
<td>70±4.4</td>
<td>80±3.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** p < 0.001
** p < 0.01
Table 20 - Sodium efflux rate constant (h$^{-1}$), intracellular sodium and potassium content (mmol/kg dry wt) and active sodium efflux on low and high salt diet in the controls

<table>
<thead>
<tr>
<th>Ouabain</th>
<th>Efflux rate constant (h$^{-1}$)</th>
<th>Intracellular electrolyte content (mmol/kg dry wt)</th>
<th>Efflux rate (mmol/kg dry wt/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Low Salt</td>
<td>0.256±0.023</td>
<td>0.106±0.006</td>
<td>0.150±0.020</td>
</tr>
<tr>
<td>High Salt</td>
<td>0.309±0.031</td>
<td>0.115±0.066</td>
<td>0.194±0.029</td>
</tr>
</tbody>
</table>
Table 21 - Body weight, blood pressure, daily urinary sodium and potassium excretion, plasma renin activity on low and high salt intakes in the normotensive relatives of hypertensive patients

<table>
<thead>
<tr>
<th>Relatives</th>
<th>Weight (Kg)</th>
<th>BP (mmHg)</th>
<th>UNa mmol/24h</th>
<th>UK ng/AI/ml/h</th>
<th>PRA 1/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low salt</td>
<td>69±2.9***</td>
<td>130±3.8</td>
<td>117±2.16</td>
<td>38±5.6***</td>
<td>63±5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>65±3.3</td>
<td>76±2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High salt</td>
<td>70±3.1</td>
<td>130±3.6</td>
<td>125±4.7</td>
<td>260±39</td>
<td>72±39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>69±3.6</td>
<td>72±3.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** p < 0.001  * p < 0.05
Table 22 - Sodium efflux rate constant (h⁻¹), intracellular sodium and potassium content (mmol/kg dry weight) and active sodium efflux on low and high salt diet in the normotensive relatives of hypertensive patients

<table>
<thead>
<tr>
<th></th>
<th>Intracellular</th>
<th>Electrolytes</th>
<th>Active sodium efflux rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium efflux rate constant (h⁻¹)</td>
<td>total</td>
<td>ouabain</td>
</tr>
<tr>
<td>Low Salt</td>
<td>0.319±0.032</td>
<td>0.127±0.010</td>
<td>0.192±0.025</td>
</tr>
<tr>
<td>High Salt</td>
<td>0.348±0.039</td>
<td>0.129±0.011</td>
<td>0.219±0.030</td>
</tr>
</tbody>
</table>
Erythrocyte Sodium Efflux Rate Constant and Cation Content

Mean erythrocyte total and ouabain sensitive sodium efflux rate constants were not significantly different in the relatives compared to normals on the normal diet. Intracellular sodium was slightly but not significantly elevated in the relatives (Table 18). The change in salt intake from low to high sodium diet caused a non-significant increase in total and ouabain sensitive efflux rate constant in both relatives and controls (Tables 20 and 22). Intracellular sodium and potassium were not changed in either group.

During both the low and high salt limbs of the study, total and ouabain sensitive sodium efflux rate constants were higher in the relatives compared to the controls. When response to both dietary changes was compared using two way analysis of variance, there was a significant difference in the response of ouabain resistant sodium efflux rate between the relatives and controls on both the low and high salt diet (p<0.05).
Discussion

This study was devised to investigate the effects of change in salt balance on erythrocyte sodium transport and in particular to determine whether active sodium pumping is inhibited by salt loading. In neither normotensive first-degree offspring of hypertensive patients nor controls with no such family history was this observed. On the contrary, there was a non-significant rise in sodium transport during salt loading but intracellular sodium did not change.

It could be argued that the change in dietary sodium intake was inadequate to produce inhibition of the sodium pump; however, the six-fold change in urinary sodium within the physiological range was sufficient to produce a significant change in blood pressure of the group as a whole (p < 0.01). Therefore, it is unlikely that inhibition of sodium transport over the period of study was missed as a result of an insufficient change in dietary salt intake.

Although it has been suggested that inhibition of membrane sodium transport by sodium loading may be an important physiological mechanism underlying hypertension (de Wardener and MacGregor 1980) there have been few studies of the
effects of change in sodium balance on membrane electrolyte transport. In the rat severe salt restriction caused a significant reduction in both total and ouabain sensitive (active) sodium efflux rate constants in the thymocyte (Bradlaugh, Heagerty, Bing et al 1984). Similarly in man, Morgan, Myers & Fitzgibbon (1981) reported red cell sodium efflux fell with salt restriction; Weissberg, West, Kendall et al (1985) found no differences between relatives and control subjects on a normal diet and demonstrated that the combined group showed a fall in sodium pump activity when moving from a low to a high salt diet. Cooper, Trevisan, Van Horn et al (1984) could not detect any change in either erythrocyte intracellular sodium or sodium-lithium countertransport. However, Gudmundsson, Herlitz, Jonsson et al (1984b) demonstrated an increase in erythrocyte sodium efflux rate constant with salt loading for four weeks, which is consistent with the findings of the present study. More recently Stokes, Monaghan, Middleton et al (1986) reported the effects of severe salt restriction (intake less than 20 mmol/day) in both normotensive and hypertensive subjects and found that blood pressure fell only in the hypertensive patients and in neither group did a change in sodium pump activity occur.
The failure to demonstrate sodium pump inhibition by salt loading is in contrast to the findings of Weissberg, West, Kendal et al (1985). However in the latter study in which the order of dietary change was not randomised, no differences in rate constants were observed on moving from normal to low salt diet. In both investigations erythrocytes were studied in their own plasma, and the discrepancy in the results obtained upon a high salt intake remain uncertain. The fact that in the study of Weissberg, West, Kendal et al (1985) no increase in sodium pump activity was observed upon severe salt restriction, and the demonstration of no change in glycoside sensitive fluxes with high salt diet in our study both argue against the presence of ouabain like factor being at work in these subjects. Therefore, it seems clear that there is no support for the hypothesis that sodium overload is responsible for the depression of erythrocyte and leucocyte sodium pump activity observed in hypertensive patients and their offspring.

Another difficulty with the hypothesis that ouabain-like inhibitors mediate the changes in the sodium transport observed in hypertensive patients and their offspring lies in the fact that other abnormalities in the ouabain resistant pathways such as sodium-potassium co-transport (Garay, Dagher, Pernollet et al 1980a) and sodium-lithium
countertransport (Canessa, Adragna, Solomon et al 1980) have been described.

Our results are in keeping with these observations since whilst diet was changed from normal ouabain resistant rate constants were significantly higher in the relatives compared to the controls irrespective of whether results upon high or low sodium intakes are considered. The apparent discrepancy of the results on normal diet may be the result of catecholamine induced depression of erythrocyte sodium pumping during the stress of the first venepuncture of these studies (Riozzi, Heagerty, Bing et al 1985). In addition, we have recently demonstrated that in relatives but not controls leucocyte ouabain insensitive sodium efflux rate constant was stimulated by the administration of a low salt diet (Heagerty, Alton, El Ashry et al 1986). The discrepancy in the results obtained in the two tissues is almost certainly attributable to the much slower rate of sodium extrusion associated with the erythrocyte. The multiple abnormalities in membrane sodium handling reported in individuals genetically prone to hypertension apparently dissociated with volume expansion suggest that there is a primary physico-chemical abnormality of the plasma membrane in hypertensive patients and their relatives (Swales 1982; Daniel and Kwan 1981). Other studies of erythrocyte membrane viscosity (Orlov and Postnov
1982) and temperature dependence of sodium fluxes (Levy, Paran, Keynan et al 1983) support the view. Further studies are required to determine whether all the abnormalities observed are determined by a biochemical change in the cell membrane but this would seem more likely than a circulating sodium inhibitor hypothesis.
CHAPTER 5

EFFECTS OF SUPPLEMENTATION WITH POTASSIUM CHLORIDE
UPON BLOOD PRESSURE AND ERYTHROCYTE SODIUM TRANSPORT
IN NORMOTENSIVE OFFSPRING OF HYPERTENSIVE PATIENTS
AND MATCHED CONTROL SUBJECTS
Introduction

As early as 1928 Addison investigated the effects of potassium salt supplementation upon blood pressure in hypertensive patients. In five subjects he noted an antihypertensive effect which was confirmed in a larger study of 45 patients by Priddle (1931). However, these patients were also taking a low salt diet. Since these early reports, there have been many other studies both in favour and against a beneficial effect of a high potassium intake. McQuarrie, Thompson and Anderson (1936) demonstrated that added potassium chloride had a protective effect on blood pressure of diabetic children receiving a high salt diet. More recently, Walker, Whelton, Saito et al (1979) demonstrated an inverse correlation between potassium and blood pressure by showing that subjects with higher blood pressure had lower urinary potassium excretion and lower potassium-creatinine ratio. Furthermore, a follow-up study revealed that blood pressure of students with a familial predisposition to hypertension fell significantly when they received a low salt diet supplemented with potassium, but there was no significant change in those without a family history. Furthermore, Skrabal, Aubock, Hortnagl et al (1980) observed an alteration of the pressure rise induced
in normotensive subjects by mental stress when potassium intake was increased from 80 to 200 mmol/day. Two studies by Parfrey and co-workers (1981a & b) showed that the combination of low dietary sodium and high potassium reduced the blood pressure of patients with mild hypertension, and the offspring of hypertensive patients. By contrast the offspring of normotensive patients responded to the diet with a non-significant rise in blood pressure.

Some studies of mild hypertensive patients have shown a modest fall in blood pressure with potassium loading (Imura, Kijima, Kikuchi et al 1981; Morgan 1982; MacGregor, Smith, Markandu et al 1982; Overlack Miller, Kolloch et al 1983; Smith, Markandu, Sagnella et al 1983; Svetkey, Yarger, Feussner et al 1987; Siani, Strazzullo, Russo et al 1987). High blood pressure is correlated more closely to low potassium intake than to high sodium intake (Dai, Kuller and Miller 1984; Reed, McGee, Yano et al 1985). Furthermore, hypokalaemic hypertensive patients receiving diuretics had a further fall in blood pressure after their potassium was increased (Kaplan, Carnegie, Raskin et al 1985). On the other hand there are two conflicting reports by Grim, Luft, Miller et al (1980) who showed that blacks have higher blood pressure than whites, despite a very low potassium intake with similar intake of sodium in the white population and Smith, Markandu, Sagnella et al (1985) who failed to observe
a fall in blood pressure of hypertensive patients receiving a 64 mmol of potassium chloride supplement to their diet.

Animal studies also suggest that potassium has a protective effect in hypertensive rats when they were intoxicated with sodium chloride (Meneely, Ball and Youmans 1957). They showed that elevated blood pressure and increased total body sodium were ameliorated by added potassium chloride. Another two studies demonstrated a correlation between blood pressure and potassium (Louis, Tabel and Speeter 1971; Dahl, Leitl and Heine 1972). These observations led Meneely and Battarbee (1976) to postulate that the high sodium, low potassium environment of civilised people, operating on a genetic substrate of susceptibility is the cardinal factor in the genesis and perpetuation of essential hypertension. A number of studies, however, have yielded opposite results. Freed, Roserman and Friedman (1953), showed that rigid restriction of dietary potassium induced a fall in the blood pressure of both normotensive and hypertensive rats. It was also shown that the addition of adequate potassium was essential for the development of DOCA/salt hypertension and Reid and Laragh (1965) demonstrated an absolute lowering of blood pressure when rats were fed with a potassium-deficient diet but a high potassium intake produced no change. However, potassium may protect hypertensive rats from vascular damage in the absence of a fall in pressure (Tobian, Lange, Ulm et al 1985).
There is some evidence that the addition of potassium can initiate vasodilatation. Overbeck, Derifield, Pamnani et al (1974); Robinson, Phillips, Wilson et al (1983) infused potassium into the forearm and noted a decrease in vascular resistance. This was attributed to the activation of the \( \text{Na}^+/\text{K}^+ \text{ATPase} \) pump because it could be abolished by the administration of ouabain (Chen, Brace, Scott et al 1972; Anderson, Winquist, Webb et al 1983). The overall impression must be similar to that drawn from studying the salt controversy: that is the higher the initial blood pressure, the greater the fall observed, with any intervention including potassium supplementation. Therefore, it was decided to investigate the effects of oral supplementation with potassium on blood pressure and erythrocyte sodium transport and to see whether the responses differed in subjects with and without a family history of hypertension.

**Subjects**

Ten subjects (5 male and 5 female), all of whom had one or more first-degree relative(s) known to have essential hypertension were studied. These were compared with ten subjects with no such family history. The characteristics of the two populations are shown in Table 23.
**Study protocol:**

On entry into the study, the subjects were weighed and their blood pressure was measured. Dietary salt intake was estimated from urinary sodium excretion of three complete 24 hour collections. A venous blood sample was drawn for plasma renin activity (PRA) and erythrocyte sodium transport studies. Subjects were then assigned to take 8 slow potassium tablets (each contains 8.06 mmol of potassium chloride) per day to double the daily potassium intake. After supplementation for fourteen days, all these tests were repeated.

Blood pressures were measured with a Hawksley random-zero sphygmomanometer. Three readings were taken in both the supine and standing positions and the mean of each set of readings was recorded. Urinary electrolytes were measured using a Corning flame photometer with a lithium internal standard. Sodium transport and PRA studies were carried out as outlined in the Methods chapter.

Statistical analysis was performed using Student's paired "t" test for within group analyses and by Student's unpaired "t" test for between group analyses. Results are expressed as mean ± SEM.
Table 23 - Shows the age, weight, height, blood pressure, urinary electrolyte excretion and plasma renin activity in normotensive offspring of hypertensive patients and matched controls.

<table>
<thead>
<tr>
<th></th>
<th>Age (kg)</th>
<th>Weight (M)</th>
<th>Height (M)</th>
<th>BP Supine in mmHg</th>
<th>UNa mmol/24 h</th>
<th>UK ng Al/ml/h</th>
<th>PRA ng Al/ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normotensive Offspring</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F:5</td>
<td>10</td>
<td>26±2.2</td>
<td>66±6</td>
<td>1.66±0.03</td>
<td>125±3</td>
<td>121±2</td>
<td>124±21</td>
</tr>
<tr>
<td>M:5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>69±4</td>
<td>79±3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Normotensive Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F:5</td>
<td>10</td>
<td>25±1.5</td>
<td>69±5</td>
<td>1.69±0.04</td>
<td>130±4</td>
<td>121±4</td>
<td>146±19</td>
</tr>
<tr>
<td>M:5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>71±6</td>
<td>77±4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 24 - Sodium efflux rate constant and intracellular electrolyte content in normotensive offspring of hypertensive patients and matched controls

<table>
<thead>
<tr>
<th>Total</th>
<th>Mean Efflux Rate Constant (h⁻¹)</th>
<th>Intracellular Electrolyte Content mmol/Kg dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ouabain</td>
<td>(Na)ᵢ</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Offspring</td>
<td>0.21 ± 0.02</td>
<td>0.11 ± 0.005</td>
</tr>
<tr>
<td>Controls</td>
<td>0.254 ± 0.04</td>
<td>0.11 ± 0.01</td>
</tr>
</tbody>
</table>
**Results**

There were no significant differences in baseline characteristics between the two groups of subjects studied (Table 23). The potassium supplementation produced no untoward effects in any of the volunteers.

**Normotensive control subjects**

Potassium supplementation significantly increased urinary potassium excretion (p<0.001, but did not influence weight, PRA or blood pressure (Table 25). Similarly, no effect was observed on any component of erythrocyte sodium transport (Table 26).

**Normotensive relatives**

In these subjects potassium supplementation significantly increased urinary potassium excretion (p<0.001) but did not affect sodium excretion (Table 27). Mean weight and blood pressure was unchanged, but there was a significant fall in PRA (p<0.05, Table 27). Potassium supplementation caused a significant rise in total erythrocyte efflux rate constant for sodium (p<0.05), which was due to a significant rise in ouabain sensitive ERC (p<0.05). Ouabain resistant ERC was
unchanged (Table 28). In addition intra-erythrocyte sodium content fell (p<0.05), but intra erythrocyte potassium content did not change. The active sodium efflux rate was not changed significantly (Table 28).

**Between group analysis**

There was no difference in mean body weight and electrolyte excretion between the groups of subjects before or after potassium supplementation. Similarly, blood pressure was unchanged by the dietary manipulation. However, mean PRA showed a different response, rising slightly in control subjects and falling significantly in the relatives. Between group analysis showed this to be significant (p<0.05).

Before the change in dietary potassium, there was no difference in any component of the erythrocyte rate constant for sodium between the controls and relatives (Tables 24 & 26). Intra-erythrocyte sodium was higher in the offspring of hypertensive patients compared to controls on normal diet, but this difference was abolished with the high potassium diet. Absolute fluxes for sodium were not different in the two groups before or after potassium supplementation.
Table 23 - Effects of potassium supplementation upon weight, urinary electrolyte excretion, PRA and blood pressure in control subjects

<table>
<thead>
<tr>
<th>WT (kg)</th>
<th>BP in mmHg</th>
<th>Urinary Na (mmol/24 h)</th>
<th>Urinary K</th>
<th>PRA (ngAl/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supine</td>
<td>Standing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Diet</td>
<td>69.6±5</td>
<td>130±4</td>
<td>121±4</td>
<td>146±19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>71±6</td>
<td>77±4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Potassium</td>
<td>69±5</td>
<td>128±2</td>
<td>132±11</td>
<td>163±29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>69±3</td>
<td>74±3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = p < 0.001
Table 26 - Effects of potassium supplementation upon erythrocyte sodium transport in normotensive control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Mean Efflux Constant (h⁻¹)</th>
<th>Intracellular Electrolyte Content [mmol/kg dry wt]</th>
<th>Active Sodium Efflux (mmol/kg dry wt/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ouabain</td>
<td>Ouabain resistant</td>
<td>Ouabain sensitive</td>
</tr>
<tr>
<td>Total</td>
<td>0.25±0.04</td>
<td>0.11±0.01</td>
<td>0.18±0.04</td>
</tr>
<tr>
<td>Normal Diet</td>
<td>0.27±0.02</td>
<td>0.01±0.01</td>
<td>0.17±0.02</td>
</tr>
<tr>
<td>High Potassium</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 27 - Effects of potassium supplementation upon weight, urinary electrolyte excretion, PRA and blood pressure in normotensive offspring of hypertensive patients

<table>
<thead>
<tr>
<th></th>
<th>Weight (Kg)</th>
<th>BP in mmHg</th>
<th>Urinary (Na+) mmol/24 h</th>
<th>Urinary (K+) ng AI/ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Supine</td>
<td>Standing</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>65.9±4.4</td>
<td>125±3</td>
<td>121±2</td>
<td>124±21</td>
</tr>
<tr>
<td>Diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>69±4</td>
<td>79±3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>67±3.8</td>
<td>130±3</td>
<td>123±3</td>
<td>157±24</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>70±3</td>
<td>71±</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05
** p < 0.001
Table 28 - Effect of potassium supplementation on erythrocyte sodium efflux rate constant and intracellular electrolyte content in normotensive offspring of hypertensive patients.

<table>
<thead>
<tr>
<th></th>
<th>Mean Efflux Rate Constant (h⁻¹)</th>
<th>Intracellular Electrolyte Content</th>
<th>Active Sodium Efflux (mmol/kg dry wt/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Ouabain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>Sensitive</td>
<td></td>
</tr>
<tr>
<td>Normal Diet</td>
<td>0.21±0.02</td>
<td>0.113±0.018</td>
<td>24±1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>224±6.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.7±0.49</td>
</tr>
<tr>
<td>High Potassium</td>
<td>0.29±0.02</td>
<td>0.17±0.02</td>
<td>20±1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>227±4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.4±0.36</td>
</tr>
</tbody>
</table>

* p < 0.05
Discussion

These results demonstrate that a potassium supplementation of 64.5 mmol per day failed to influence blood pressure but, stimulated ouabain sensitive sodium efflux rate constant in the normotensive offspring of hypertensive patients, whilst not influencing sodium transport in control subjects. Likewise, plasma renin activity was influenced differently in the two populations, remaining unchanged in controls and falling in the relatives. Urinary potassium excretion was used as an index of compliance and rose in both groups of subjects. Other sources of potassium excretion such as faecal loss were not monitored, but it was apparent that compliance was good.

The failure to influence blood pressure is consistent with other studies in similar populations (Freed, Roserman and Friedman 1953; Smith, Markandu, Sagnella et al 1985). However, significant stimulation of the sodium pump should have been associated with a fall in pressure (de Wardener and MacGregor 1982) which was not observed, although sodium pump activity did rise in the offspring of hypertensive patients.
Plasma renin activity also changed in a different fashion, dependent upon the family history of the subject. A fall in PRA is in agreement with studies performed by others (Bull, Hillman, Cannon et al 1966; Veyrat, Brunner, Grandchamp et al 1967; Maebashi, Miura and Yoshinaga 1968; Flamenbaum, Kotchen, Nagle et al 1973). However, sodium excretion was unchanged, overall making interpretation somewhat perplexing.

The differential effects of the supplementation upon sodium transport are worthy of note. If it is assumed that any changes are attributable to a direct influence of potassium ions upon the Na\(^+\)/K\(^+\)ATPase enzyme, then the cells of subjects genetically prone to essential hypertension behaved differently to those from subjects with no such predisposition. Similarly, it is possible that cells from offspring can recruit more active pump sites when challenged, compared to control subjects. Without information from other studies such as ouabain binding for example, any interpretation must be purely speculative.
CHAPTER 6

EFFECTS OF CHANGING FAT INTAKE ON BLOOD
PRESSURE AND ERYTHROCYTE SODIUM TRANSPORT IN
NORMOTENSIVE OFFSPRING OF HYPERTENSIVE
SUBJECTS AND MATCHED CONTROLS
Introduction

Initial reports of disturbances of plasma membrane function in cells of hypertensive patients (Swales 1982; 1983) were confined to the handling of univalent cations such as sodium (Edmondson, Thomas, Hilton et al 1975 and Heagerty, Milner, Bing et al 1983). However, others have demonstrated disturbances in calcium binding and efflux (Wei, Janis and Daniel 1976; Postnov, Orlov and Pokudin 1979). Some of these mechanisms have been found to be disordered in the normotensive offspring of hypertensive patients (Meyer, Garay, Nazaret et al 1981; Heagerty, Milner, Bing et al 1982 and Woods, Falk, Pittman et al 1982). Moreover, similar abnormalities have been demonstrated in obesity (Deluise, Blackburn and Flier 1980). It has been suggested that in the hypertensives, there may be markers of a genetically determined alteration in the physicochemical structure of the plasma membrane (Postnov and Orlov 1984; Heagerty, Rizzi, Brand et al 1985). The site of this membrane abnormality may well lie in the lipid fraction (Swales 1982; Heagerty, Rizzi, Brand et al 1985). Thus erythrocyte membrane microviscosity (Montenay-Garestier, Aragon, Devynck et al 1981) and sialic acid content (Reznikova, Adler, Postnov 1984) are deranged in hypertension and there is an inverse relationship between platelet and adipose tissue
linoleic acid and blood pressure (Wood, Butler, Riemersma et al 1984).

The fatty acid content of membrane lipids determines the composition and activity of many complex phospholipids (Kishimoto, Takai, Mori et al 1980) and these in turn influence the membrane handling processes such as Na$^+/K^+$ ATPase activity, ouabain resistant processes and the Ca$^{++}$/Mg$^{++}$ ATPase pump (Jorgensen 1975; Roelofsen 1981).

Recently, a great deal of interest has been aroused by the observations that blood pressure can be lowered by changing from an omnivore to a vegetarian diet (Rouse, Beilin, Armstrong et al 1983), a manoeuvre that will alter membrane fatty acid composition (Sanders, Ellis, Dickerson 1978). The exact component of a vegetarian diet that lowers blood pressure is unclear, but an increased polyunsaturated fat intake has been implicated (Puska, Iacono, Nissinen et al 1983). It was decided therefore to study whether increasing the intake of a polyunsaturated lipid (linoleic acid) will change the blood pressure and/or the membrane sodium transport in the normotensive offspring of hypertensives and matched controls.
Subjects and Methods

Fourteen healthy normotensive subjects were studied (7 male and 7 female), all with at least one hypertensive parent. These subjects were compared with sixteen normotensive controls (8 male and 8 female) with no family history of hypertension in siblings or parents. All subjects were Caucasian and there were no significant differences in age, weight, plasma renin activity or blood pressure between the groups. All subjects were omnivorous.

Study Protocol

The trial design was double-blind and randomized. Upon recruitment, volunteers were weighed and their height, supine and standing blood pressures were recorded. Blood pressures were measured using a Hawksley Random Zero sphygmomanometer, three readings were taken in both the supine and standing positions and the means recorded. All subjects provided three successive 24 hour urine collections for estimation of sodium and potassium excretion. Subjects were then instructed to continue their normal diet and randomized to receive either linoleic acid or placebo supplements.
Linoleic acid was administered as eight Safflower seed oil capsules per day. Each capsule contained 500 mg of oil of which 72% was linoleic acid, a polyunsaturated fatty acid. It was calculated that this would increase average daily linoleic acid intake by approximately 40%. Placebo capsules were identical to active supplements but contained paraffin. Subjects continued taking capsules for twenty-eight days. At the end of the time they were weighed, their blood pressure was remeasured and venous blood was collected for erythrocyte sodium transport studies as described in Chapter 2. A 24 hour urine collection was made during the last day of the diet period for estimation of urinary sodium and potassium excretion. Subjects then had a 28 day washout period before being crossed over to the other capsules for a further twenty-eight days, after which the studies were repeated. All subjects gave full informed consent and the study was approved by the local Ethical Committee.

**Statistical Analysis**

This was performed using a Student's paired "t" test on the data obtained at the end of the active (linoleic acid) and placebo (paraffin) dietary periods. The response to dietary change of the two groups on active and placebo dietary periods was compared using analysis of variance.
Results

There was no significant difference in the baseline characteristics (height, weight, blood pressure and urine electrolyte excretion) between the relatives and the controls on their normal diets (Table 29). All of the subjects recruited to this study completed both arms of the protocol. Some volunteers felt bloated on the Safflower seed oil capsules but otherwise suffered no ill effects. Furthermore, no subject noted any change in bowel habit during the placebo period.

Body weight and urinary electrolyte excretion

Some of the subjects reported a rise in body weight when on the active capsules but overall there was no significant change in mean weight between the dietary periods. Similarly, there was no significant change in either urinary sodium or potassium excretion (Tables 31 and 33).

Blood pressure

Both the systolic and diastolic blood pressures of the controls fell during the Safflower oil dietary phase but blood pressure appeared to be non-significantly higher in the relatives during linoleic acid supplementation. Two way
Table 29 - Baseline characteristics: mean weights, blood pressure, urinary electrolyte excretion and PRA in control subjects and relatives on normal diet.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>BP (mm Hg)</th>
<th>UNa</th>
<th>UK</th>
<th>PRA (ng Al/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Supine</td>
<td>Standing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>M:8</td>
<td>26±1.7</td>
<td>67.2±3.9</td>
<td>133±4</td>
<td>123±4</td>
<td>151±9</td>
</tr>
<tr>
<td>F:8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>69±3</td>
<td>80±3</td>
<td></td>
</tr>
</tbody>
</table>

| Relatives |
| 14  | M:7 | 25±1.5      | 67.5±3.5   | 128±2 | 121±2 | 162±17 | 77±6 | 6.17±0.83 |
|     |     |             |            |      |      |      |      |         |
|     |     |             |            | 69±4 | 77±3 |
Table 30 - The sodium efflux rate constant, intracellular electrolyte and active sodium efflux rate in controls and relatives on normal diet

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Relatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean efflux rate constant (hr⁻¹)</td>
<td>Ouabain Total 0.39±0.028 0.137±0.011 0.255±0.002</td>
<td>Ouabain Total 0.43±0.027 0.153±0.011 0.281±0.024</td>
</tr>
<tr>
<td></td>
<td>Ouabain Resistant 0.137±0.011</td>
<td>Ouabain Resistant 0.153±0.011</td>
</tr>
<tr>
<td></td>
<td>Ouabain Sensitive 0.255±0.002</td>
<td>Ouabain Sensitive 0.281±0.024</td>
</tr>
<tr>
<td>Total</td>
<td>19.16±1.13</td>
<td>19.32±1.07</td>
</tr>
<tr>
<td>(Na)i</td>
<td>232.9±7.95</td>
<td>232.3±5.61</td>
</tr>
<tr>
<td>(K)i</td>
<td>5.0±0.51</td>
<td>5.27±0.4</td>
</tr>
<tr>
<td>Intracellular electrolyte content (mmol/Kg dry wt)</td>
<td>Active Sodium efflux rate (mmol/kg dry wt/h)</td>
<td></td>
</tr>
</tbody>
</table>
Table 31 - Effects of dietary supplementation with Safflower oil (linoleic acid) on body weight, BP, urinary electrolyte and PRA of the control subjects

<table>
<thead>
<tr>
<th>Dietary Period</th>
<th>Wt (kg)</th>
<th>BP (mm Hg)</th>
<th>UNa (mmol/24 h)</th>
<th>UK (ng Al/ml/h)</th>
<th>PRA (ng Al/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Supine</td>
<td>Standing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>67.7±3.9</td>
<td>133±4</td>
<td>121±4</td>
<td>142±14</td>
<td>75±5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>67±3</td>
<td>75±3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safflower oil</td>
<td>67.5±3.8</td>
<td>128±4 *</td>
<td>118±4</td>
<td>129±15</td>
<td>70±6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>68±2</td>
<td>72±2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05
Table 32 - Effects of Safflower oil supplementation (Linoleic acid) on sodium efflux rate constants, intracellular electrolytes and active sodium efflux in the control subjects

<table>
<thead>
<tr>
<th></th>
<th>Mean efflux rate constant (h⁻¹)</th>
<th>Intracellular electrolyte content (mmol/kg dry weight)</th>
<th>Active Sodium efflux rate (mmol/kg dry wt/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Ouabain Resistant Ouabain Sensitive</td>
<td>(Na)i</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.395±0.03</td>
<td>0.153±0.01 0.241±0.03</td>
<td>18.59±1.11</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>0.447±0.2</td>
<td>0.148±0.01 0.297±0.02</td>
<td>18.13±0.81</td>
</tr>
</tbody>
</table>
Table 33 - Effects of dietary supplementation with Safflower oil (Linoleic acid) on the weight, BP, urinary electrolytes and PRA of relatives

<table>
<thead>
<tr>
<th>Dietary Period</th>
<th>Weight (kg)</th>
<th>BP (mm Hg)</th>
<th>UNa (mmol/24 h)</th>
<th>UK (ng AI/ml/ h)</th>
<th>PRA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supine</td>
<td>Standing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>68±3.6</td>
<td>126±4</td>
<td>116±3</td>
<td>168±21</td>
<td>77±7</td>
</tr>
<tr>
<td></td>
<td>66±3</td>
<td>63±3</td>
<td></td>
<td></td>
<td>4.96±0.58</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>68±3.7</td>
<td>131±3</td>
<td>131±3</td>
<td>134±19</td>
<td>70±7</td>
</tr>
<tr>
<td></td>
<td>67±3</td>
<td>67±3</td>
<td></td>
<td></td>
<td>9.6±1.16 *</td>
</tr>
</tbody>
</table>

* p < 0.05
Table 34 - Effects of Safflower oil supplementation (linoleic acid) on sodium efflux rate constants, intracellular electrolytes and active sodium efflux in relative group.

<table>
<thead>
<tr>
<th>Dietary Period</th>
<th>Mean Ouabain (^{-1}) Efflux Rate Constants (h(^{-1}))</th>
<th>Intracellular Electrolyte Content (mmol/kg dry wt)</th>
<th>Active Sodium Efflux Rate (mmol/kg dry wt/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.454±0.012</td>
<td>0.153±0.007</td>
<td>0.302±0.015</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>0.414±0.036</td>
<td>0.148±0.01</td>
<td>0.265±0.032</td>
</tr>
</tbody>
</table>
analysis of variance showed that the blood pressure response to dietary change was significantly different in the controls compared to the relatives ($p<0.05$).

Interestingly, plasma renin activity (PRA) was significantly increased in the relatives during the active phase ($p<0.05$) but was unchanged in the control subjects (Tables 31 and 33). There was no evidence of an order effect in either group of subjects (Hills and Armitage 1979). When the response of relatives and normals to Safflower oil supplement was compared there was a significant difference in supine blood pressure ($P<0.05$). In addition there was a significant difference in plasma renin response ($P<0.01$).

**Erythrocyte sodium efflux rate constant and cation content**

On the normal diet, mean total and ouabain sensitive erythrocyte efflux rate constants and intracellular sodium were not significantly different in the relatives compared to the normals (Table 30). Dietary supplementation with Safflower oil produced a non-significant rise in both total and ouabain sensitive efflux rate constants in the normals (Table 32) with no change in the relatives (Table 34). There was no change in intracellular sodium in either the normals or the relatives. When the response of the relatives and normals to Safflower oil supplement was
compared there was an almost significant difference in the response of sodium efflux rate constant observed \((p = 0.05)\). There was also a significant difference in ouabain sensitive sodium efflux rate \((p < 0.05)\).

**Erythrocyte membrane fatty acid composition**

The coefficients of variation for the erythrocyte fatty acid composition calculated from 10 subjects were as follows:

- Palmitic acid (16:0) 4.5% - Stearic acid (18:0) 4.9% - Oleic acid (18:1) 5.1% - Linoleic acid (18:2) 7.7% - Arachidonic acid (20:4) 9.4%.

In the preliminary studies, linoleic acid values were measured before and 28 days after the ingestion of the Safflower seed oil capsules to ensure that the wash-out period was long enough. There was no significant difference between the linoleic acid content at baseline and after the washout period \((11.73\pm0.3 \text{ v. } 11.8\pm0.3\%, p = 0.76)\).

During the study, there were no significant changes in the saturated fatty acids (palmitic and stearic) in the controls but palmitic acid levels decreased in the relatives during the active phase \((p < 0.05)\). Similarly, the levels of oleic and arachidonic acid were unchanged in the controls but oleic
### Table 33- Mean percentage erythrocyte fatty acid content during treatment with placebo and Safflower seed oil.

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS</th>
<th>PLACEBO</th>
<th>SAFFLOWER OIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid 16:0</td>
<td>24.46±0.39</td>
<td>24.75±0.41</td>
<td></td>
</tr>
<tr>
<td>Stearic acid 18:0</td>
<td>22.93±0.24</td>
<td>23.06±0.24</td>
<td></td>
</tr>
<tr>
<td>Oleic acid 18:1</td>
<td>20.37±0.29</td>
<td>20.15±0.31</td>
<td></td>
</tr>
<tr>
<td>Linoleic acid 18:2</td>
<td>16.54±0.47</td>
<td>16.93±0.37</td>
<td></td>
</tr>
<tr>
<td>Arachidonic acid 20:4</td>
<td>15.15±0.31</td>
<td>14.98±0.28</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>RELATIVES</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid 16:0</td>
<td>25.08±0.34</td>
<td>24.18±0.46</td>
<td>*</td>
</tr>
<tr>
<td>Stearic acid 18:0</td>
<td>22.57±0.27</td>
<td>22.90±0.24</td>
<td></td>
</tr>
<tr>
<td>Oleic acid 18:1</td>
<td>20.74±0.24</td>
<td>20.31±0.26</td>
<td>*</td>
</tr>
<tr>
<td>Linoleic acid 18:2</td>
<td>16.70±0.36</td>
<td>16.71±0.30</td>
<td></td>
</tr>
<tr>
<td>Arachidonic acid 20:4</td>
<td>14.43±0.24</td>
<td>15.16±0.42</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05
acid levels fell significantly in the relatives ($p < 0.05$).
The content of linoleic acid increased in controls, but not the relatives, during the active treatment (Table 35).

**Discussion**

In normotensive first degree offspring of hypertensive subjects, this dietary change had no influence upon total ouabain resistant or ouabain sensitive rate constants for sodium, nor was intra-erythrocyte sodium or potassium changed. Blood pressure was not significantly altered: the only change of significance within the group was a rise in plasma renin activity. Conversely, in the control subjects, there were small falls in blood pressure in both the lying and standing positions, with that of supine systolic blood pressure achieving significance. However intracellular sodium was not altered and the small rise in the total and ouabain sensitive efflux rate constants failed to achieve significance.

Using the analysis of variance, the response in supine systolic blood pressure was significantly different in controls compared to relatives as was the change in plasma renin activity, total efflux rate constant and ouabain sensitive sodium pump activity. These changes were achieved using a dietary manipulation that left sodium intake
unaltered. The difference in response between the two groups would imply that this dietary manipulation has different effects on the two populations.

Whilst, it has been known for many years that vegetarians have a lower blood pressure than omnivores (Donaldson 1926), the reason for this is unclear. There are a large number of differences in a pure vegetarian diet in terms of nutrients and vitamins (Rouse and Beilin 1984) compared to a omnivore diet. One of the largest differences between vegetarian and omnivore diets is the proportion of polyunsaturated fat ingested (Sanders, Ellis and Dickerson 1978). Recent studies have suggested that an increase in the polyunsaturated/saturated fat ratio of an omnivore diet can lower blood pressure (Puska, Iacono, Nissinen et al 1983). Nevertheless, the precise dietary change that affects this is uncertain and existing studies have failed to identify the factors responsible. In this study, one variable was changed by increasing dietary linoleic acid and by doing so different effects were observed in erythrocyte membranes of normotensive control subjects and the normotensive offspring of hypertensive patients. In the control subjects, the changes in blood pressure observed were similar to those seen on a vegetarian diet (Rouse, Beilin, Armstrong et al 1983). The amount of fat used was small and did not appear to affect the volunteers adversely. It was planned to use a
rise in erythrocyte membrane linoleic acid as an index of fat incorporation into the plasma membrane; this tissue is used because these cells are relatively free of organelles membrane whose lipid composition might differ from that of the plasma membrane itself. It is unlikely that the long half life of these cells might complicate these studies as the fat composition of cell membranes varies with dietary intake (Kernoff, Willis, Stone et al 1977) and a four week washout period between two dietary periods would have been sufficient for the membranes to adjust from the dietary change (Lorenz, Spengler, Fischer et al 1983).

In the controls the amount of linoleic acid incorporated was small and the change failed to achieve significance. However, a larger group of 22 controls receiving the same regime did demonstrate a rise in erythrocyte membrane linoleic acid (Heagerty, Ollerenshaw, Robertson, et al 1986). By contrast a quite different pattern was observed in the relatives of hypertensive patients where significant falls in membrane palmitic and oleic acid were observed (Table 35).

These changes although small were sufficient to cause a significant difference in total and ouabain sensitive response of the erythrocyte efflux rate constants for sodium between the relatives and control subjects. However
absolute efflux rates for sodium were unchanged and contrary to previous studies (Heagerty, Ollershaw, Robertson et al 1986a), it would appear that in this cell there has been no influence upon sodium influx. The second question raised by this study is that although linoleic acid clearly changes the membrane characteristics for sodium handling, the blood pressure lowering effects may have been caused by other influences of the lipid on circulatory homeostasis. In this regard, it is interesting to note that the supplementation of a normal Western diet with cod liver oil which is enriched in long chain w-3 polyunsaturated fatty acids also lowered blood pressure but did not influence erythrocyte sodium cotransport or countertransport characteristics (Lorenz, Spengler, Fischer et al 1983).

Whilst significant changes were observed in control subjects with respect to blood pressure the converse was true in the offspring of hypertensives where no change was observed. This would imply a differential influence of the dietary change on the plasma membrane. Two possible explanations should be entertained: the first is that the membrane of these people differ from control subjects due to a genetic disturbance of the physicochemical structure of the cell membrane (Heagerty, Milner, Bing et al 1982; Bing, Heagerty, Thurston et al 1986). The second is that the membrane handling of fatty acids is defective. Again, there is some
evidence to support this contention (Singer, Voigt, Goedicke 1982: Singer, Jaeger, Voigt et al 1984). What does seem clear, however, is that the dietary change has been unable to effect an alteration in blood pressure downwards and close scrutiny of the results would suggest a small pressor effect was induced. The properties of complex phospholipids are dependent on the fatty acid make-up of the membrane (Roelofsen, 1981); thus the dietary supplementation may well have altered their metabolism in different ways in the two sets of subjects. Recent evidence has suggested that phospholipids play an important role in the regulation of intracellular calcium (Tokumura, Mostafa, Nelson et al 1985), and may be important in the regulation of vascular contractility. It is also possible to postulate a cellular action of dietary lipids upon blood pressure via an effect on prostaglandin synthesis (Puska, Iacono, Niissinen et al 1983). Thus, either an inherited abnormality of membrane structure or a defect on the handling of fatty acids might account for different populations of prostaglandin subclasses being produced and different effects on blood pressure being observed. In control subjects, clearly, a small change in dietary fat intake has caused a fall in blood pressure, although this was only modest. The amount of linoleic acid incorporated into the cell membrane was very small and large quantities might have produced a greater effect.
It has been calculated that an equivalent fall in blood pressure of the United Kingdom population would be as beneficial as treating all patients with a diastolic blood pressure of 105 mmHg or more (Rose 1981). However, the disappointing observation of a negative effect upon the offspring of hypertensive patients who themselves are at increased risk of developing high blood pressure later in life, means that this dietary manipulation must be viewed with caution when tried on a population at genetic risk. Further studies must now follow where linoleic acid supplementation to a greater degree is accompanied by studies of sodium transport and changes on blood pressure, particularly in the normotensive offspring to see whether the pressor effect is genuine or whether increasing the supplementation will overcome a defect in lipid handling and ultimately lead to an anti-hypertensive effect.
CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS
The experiments described in this thesis have demonstrated that patients with untreated essential hypertension have significantly increased intra-erythrocyte sodium. Efflux rate constant for sodium was significantly increased although absolute efflux rates were not significantly different. Similar trends were observed in normotensive offspring of essential hypertensive patients, but no component attained statistical levels of significance.

A number of dietary manipulations were subsequently undertaken. In the first, an attempt was made to expand plasma volume using high salt intake to try and induce inhibition of glycoside sensitive efflux. This was not observed; in addition a reduction of sodium efflux rate constant was observed when salt intake was lowered. Furthermore, a qualitative difference in the response to dietary change was observed when control subjects with no family history of hypertension were compared and those with one or more first degree hypertensive relatives.

Similarly, supplementation of the normal omnivore diet with potassium failed to influence blood pressure but again demonstrated qualitative differences between the response in erythrocyte sodium transport in control subjects and that seen in offspring of hypertensive patients.
In view of the possibility of an intrinsic membrane abnormality underlying these qualitative differences, an attempt was made to alter sodium transport in erythrocyes by supplementing the diet with polyunsaturated fat (linoleic acid) which was subsequently demonstrated to be incorporated into the erythrocyte membrane whilst small changes in sodium transport were observed: these did not attain statistical significance in either the offspring of hypertensive subjects or in controls. However, the control subjects did show a fall in systolic blood pressure.

The erythrocyte is comparatively slow with respect to transmembrane sodium flux. In all the experiments it is conceivable that there were residual influences of plasma hormones present at the time of venesection. Indeed, final fluxes were performed in plasma drawn at the time when the erythrocytes were collected and saved. This may in itself improve the chances of accurate measurements and especially if as has been postulated a humoral substance is present in hypertension, my experiments should have detected this.

However, any other hormone present in excess might still have an influence upon the ultimate measurements performed. Thus, one must be cautious in drawing conclusions when interpreting my findings here. What does seem clear
however, is that the small changes that have been observed in these experiments are compatible with those that have been reported in the literature (Swales 1982) but appear to be too small and ill-defined to be contributing on a major basis to blood pressure generation even if they are generated by an intrinsic disturbance of membrane function or due to humoral factors or even a combination of both.

Despite the uncertainty with regard to contributions from the plasma factors or the membrane itself, incubation of cells in plasma would appear to negate the contributions of ouabain like factors in the generation of essential hypertension because significant depression of the sodium pump was not observed in essential hypertensive patients. Whilst this would appear to be the most attractive conclusion, one must be cautious in as much as I have already indicated that the well established slow turnover of sodium might mean that the binding of any inhibitor would have to be on a prolonged basis to show a significant change in sodium movements, therefore merely exposing cells after many washes to plasma for the short period of time during which the sodium flux experiments were performed, may not be sufficient to re-institute any inhibitory effects of a plasma factor.
My dietary manipulations however did reveal changes in ouabain resistant fluxes and a glycoside-like factor cannot be implicated in these changes. Indeed, these pathways do not appear to be hormone sensitive and taken in conjunction with a number of other reports of abnormalities of sodium co-transport and counter-transport in essential hypertension it would appear unlikely that a glycoside-like factor can be the sole explanation for the disturbances of sodium transport observed in this condition.

The selection of individuals for each study was meticulously performed, nevertheless the criticism could be made that in some experiments the number of subjects with a family history of raised blood pressure studied was somewhat small. This becomes especially relevant when one considered that even if one has both parents with essential hypertension one only has a 30% of developing the disease oneself, therefore amongst 10 subjects with a family history of the disease one might only expect one or two to develop a clinically overt essential hypertension.

Qualitative differences in sodium transport induced by dietary changes therefore in a group of this size make the theory that the subjects have a genetic predisposition to structural deformity of the cell membrane attractive. As
has always been maintained by the proponents of a humoral hypothesis, experiments performed upon blood cells must be analogous to the way sodium is handled in resistance arterioles. Presently no experiments have been performed comparing erythrocyte sodium handling with that of such smooth muscle cells. Indeed, the only tangible evidence that there is a similarity comes from work on leucocytes (Aalkjaer, Heagerty, Parvin et al 1986). Nevertheless, the data presented here are inconsistent with a single humoral like factor having a profound effect upon the membrane of sufficient magnitude to cause changes in membrane potential and contractility in vascular tissue.

It becomes increasingly attractive therefore to invoke an alternative hypothesis: there is a genetic abnormality of physicochemical structure and function of the plasma membrane of which these experiments and their measurements offer just one glimpse.
APPENDIX
**Apparatus**

Shaking Water Bath
Coolspin Centrifuge
Sorvall (R.C.S.B.) refrigerated Superspeed Centrifuge
Eppendorf Microcentrifuge
Perkin-Elmer Gas Liquid Gas Chromatograph (Model FL7)
Balances - Mettler ME22
Mettler BE22
100°C oven
Gamma Counter
Stop-Clock

**Materials**

Vacutainers (10 ml lithium heparin)
Needles (19 gauge)
Aluminium foil
Surgical gloves
12 ml conical plastic tubes
Reagent Test tubes
LP3 tubes
Sterilin containers
Pasteur Suction Pipettes
Pipettes (0.2ml, 1 ml and 5 ml)

Grant Instruments, Cambridge, U.K.
MSE Instruments, Pisons, Crawley, UK.
Sorvall Instruments
Du Pont Co., Wilmington, U.S.A.
R.W. Jennings, Nottingham, U.K.
Perkin-Elmer Ltd., Beaconsfield, Bucks, U.K.
Fisons, Galenkamp, Loughborough, UK.
Jencons Scientific Ltd., Bedfordshire, U.K.
Hewlett-Packard Ltd., U.K.
British Clock Systems, U.K.
Becton Dickinson, Rutherford, New Jersey, U.S.A.
Becton Dickinson, Rutherford, New Jersey, U.S.A.
Denomaid, U.K.
Johnson and Johnson, U.K.
Sarstedt, West Germany.
Sarstedt, West Germany.
Luckham Ltd Tubes, Sussex, U.K.
Sterilin, Hampshire, U.K.
Bilbase, Daventry, Warwickshire, U.K.
Gilson Anachem Ltd., Luton, Ltd.
Sodium chloride Amersham International U.K.
Patty acid methyl ester standards (FAMES) Fisons, Gallenkamp, Loughborough, U.K.
Magnesium Chloride (Analar) Fisons " "
Calcium Chloride (anhydrous) Fisons " "
Chloroform (spectrometer grade) Fisons " "
Hexane (spectrometer grade) Fisons " "

**SOLUTIONS**

**Incubation Buffer** - NaCl 104 mmol/l, KCl 5 mmol/l, NaHCO₃ 25 mmol/l, NaH₂PO₄ 1.65 mmol/l, Na₂PO₄ 9.35 mmol/l and glucose 10 mmol/l, adjusted to pH 7.40 by gassing with a mixture of O₂-CO₂ (95:5 v.v) at 37°C at atmosphere pressure.

**Saline/EDTA Solution** - 8.9 g sodium chloride + 0.04 g potassium ethylene diamine tetraacetate dipotassium salt (EDTA-BHT) in distilled water.

**Isopropanol/BHT Solution** - 0.91 g Butylated hydroxytoluene (Sigma) in 100 ml of propan-2-ol (Spectrometer grade, Fisons).

**Sodium Methoxide Solution** - 1.08 g sodium methoxide (Fisons) in 10 mls methanol (Analar, Fisons).
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REFERENCES


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Title: Studies of Erythrocyte Sodium Transport in Essential Hypertension
Author: Ahmed El-Ashry

This thesis reports studies of erythrocyte membrane sodium handling in untreated patients with essential hypertension, their normotensive offspring and normotensive subjects with no family history of hypertension. Normotensive subjects were studied on their normal diet and after dietary manipulation of sodium, potassium and fat intake.

Patients with essential hypertension had a significantly increased erythrocyte sodium and total sodium efflux rate constant but the absolute flux rate was not significantly different. Similar trends were observed in the normotensive offspring of hypertensive patients but none of the components achieved statistical significance. In the normotensive subjects a number of dietary manipulations were undertaken. The first involved an attempt to expand plasma volume using a high salt intake to try and induce inhibition of the glycoside sensitive sodium efflux. -This was not observed: in addition a reduction of sodium efflux rate constant was observed when the salt intake was lowered. Furthermore, there was a qualitative difference in the pattern of response to dietary change when control subjects with no family history of hypertension were compared to those subjects with one or more first degree hypertensive relatives. Similarly, supplementation of the normal omnivore diet with potassium revealed a qualitative difference in response in erythrocyte sodium transport in the controls and that seen in offspring of hypertensive patients.

In view of the possibility of an intrinsic membrane abnormality underlying these qualitative differences, an attempt was made to change sodium membrane handling by supplementing the diet with polyunsaturated fat (linoleic acid) which was subsequently shown to be incorporated into the erythrocyte membrane. Small changes in sodium transport were
observed but these did not achieve statistical significance in either the offspring of hypertensive subjects or controls. However, a significant fall in systolic blood pressure was observed in the control subjects.

These studies are inconsistent with a single humoral factor having a profound effect upon membrane sodium handling but are in keeping with an alternative hypothesis that there is a genetic abnormality of physico-chemical structure and function of the plasma membrane in essential hypertension.