

Studies of Leucocyte Sodium Transport

in Essential Hypertension

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I dedicate this thesis to my father, mother, Beverly and Giles.

PREFACE

Essential hypertension is the most known reversible cause of cardiovascular mortality and morbidity and yet the actual cause of this disease remains elusive. This frustrating state of affairs is partly due to the difficulty in studying human tissue pertinent to hypertension and also because it has only been possible recently to develop refined techniques to take a detailed look at possible pathophysiological mechanisms underlying the problem. This thesis reports studies that I have undertaken into the role of cell membrane handling of sodium in essential hypertensive patients and their offspring, in an attempt to examine possible mechanisms that might contribute to raised blood pressure. It deals with the historical links between salt ingestion and essential hypertension as well as the evidence for and against dietary sodium manipulations being at fault or beneficial in blood pressure elevation and amelioration, as well as the results of my experiments using peripheral blood leucocytes.

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Chapter 1

Dietary Salt and Hypertension: Historical Review

An association between salt ingestion and disease(s) of the kidney and cardiovascular system has been known since the writings of the Chinese (Swales 1975); indeed, eating a cupful of salt was a Chinese method of committing suicide. However, specific proof of cause and effect with regard to essential hypertension has defied description for a variety of reasons. Primarily, it has only been since the work of Mahomed (1879) that the entity of high blood pressure not associated with renal disease was recognised and subsequently labelled essential hypertension. Many of the early experiments on salt and hypertension failed to discriminate between the two processes, and whilst some patients with chronic renal failure will benefit from sodium restriction, this is a far cry from explaining the elevation of blood pressure seen in essential hypertension. Second, the chemical make-up of salt might have been known, but biochemical measurements of sodium and chloride were not available until one hundred years ago. Indeed chloride was believed by many workers to be the main ion causing raised blood pressure for many years into this century. The evidence linking sodium to essential hypertension is based on anecdote until 1902 and it is only over the last 80 years that the hypothesis has received methodical testing. Evidence is drawn from three sources: amelioration of blood pressure by dietary salt restriction, attempts to raise pressure by extremes of salt-loading and studies of primitive cultures which consume a fraction of the sodium contained in the diet of Western Man.

Amelioration of Blood Pressure by Reduction of Dietary Sodium Ingestion

The belief that the oedema of cardiac failure was due to the retention of sodium had been eminently stated by Baynard

and Floyer (1722) "salts creep with the chyle into the blood and have no way out but by the urine." It was therefore given to treating "brine logged" patients by a variety of fluid restricting regimes. Probably influenced by such traditions the milk diet was introduced in 1831 by Chrestien, whereupon severely ill patients were limited to two pints of milk per day. He reported dramatic results with patients with anasarca. Subsequently his pupil Karrell (1865) tested the regime further and again reported remarkable results in oedema due to heart disease, and even advanced renal failure. Although neither they nor the succeeding physicians using the therapy were aware that success was probably based on the first therapeutic use of combined salt and water restriction.

During the middle of the nineteenth century workers began to measure urinary excretion of chloride, and later sodium. Reduction in the excretion of both was reported in febrile illnesses (Salkowski 1871) and subsequently the first report appeared of sodium and chloride retention being linked to the oedema of heart failure (Koranyi 1898). By 1901 workers were convinced that retention of chloride was responsible for oedema states: Achard and Loeper (1901) studied chloride excretion in many diseases and confirmed previous results. It was but a small step to next try and restrict chloride intake as a therapeutic manoeuvre. Widal and Jaral (1903) studied patients with renal failure and found that adding salt to the diet produced peripheral, pulmonary and cerebral oedema, and limitation resolved the oedema states. With the ability to measure blood pressure now available, trials of such dietary manipulations could be performed in hypertension. The first of these was reported by Ambard and Beaujard (1904), who compared the effects of 14.5 gm (247 mmol) and 3 gm (51 mmol)

salt diets in six hypertensive patients. There were small reductions in blood pressure on the low salt diet and increases on the high salt intake. The patients had renal failure, or valvular heart disease, and one was an alcoholic and probably in liver failure. Again the cause of success was attributed by the authors to restriction of the chloride ion not sodium: dietary salt reduction was merely the instrument by which body chloride was lowered. Moreover, although this study is often quoted as the first positive evidence that salt restriction lowers pressure in essential hypertension, this was not so: the patients for the most part had renal disease and indeed were described as "Brightiques". Further the basis of the low salt intake was the milk diet: thus a variety of other dietary constituents was also changed. Overall trial design was poor (as might be expected at this stage in the evolution of knowledge), with no control period and no set duration of therapy. However other workers adopted similar regimes with good results (Laufer 1904, Bayer 1907). The criticism was soon levelled that the patients studied were not suffering from essential hypertension, and there followed a report of salt restriction in six patients with no renal disease in whom the response was good (Blurn 1920). This study however contains no diastolic blood pressure readings and no control periods. The first study to address itself to such problems appeared shortly after Bayer's work was reported. Lowenstein (1908) believed the therapeutic approach to hypertension included bedrest and took this into account when looking at changes in salt intake in renal hypertension. Again, like Ambard and Beaujard, he believed that the chloride ion was the essential factor responsible, but although blood pressure fell in 8 out of 10 of his patients on a low salt intake,

he could not establish a firm correlation between chloride excretion and blood pressure fall. The control period was only 5 days and again other dietary constituents were also changed. The response outside France was unenthusiastic; and elsewhere the experience in the treatment of renal hypertension with salt restriction was not as good (Combe 1905; Loeb 1905) for reasons that have only subsequently become apparent with a knowledge of the diverse handling of sodium and water that can occur in renal failure. Thus not all patients with renal failure are volume-expanded as some waste sodium secondary to impaired tubular sodium reabsorption. Outside France little attention was paid to salt-poor diets for the treatment of blood pressure. Indeed, Frederick Allen (Allen and Sherill 1922), the most ardent supporter of salt restriction in America during the early twentieth century commented: "In discussions of the subject of hypertension before the American Medical Association following the papers of Miller and of Foster not a single speaker advocated chloride restriction, as far as the published records show". No good study addressed itself to whether essential, as opposed to renal hypertension, could be influenced by changes in salt intake. Because of the assertion that the chloride ion was crucial to fluid balance, a belief firmly held by Ambard and other prominent French workers, the sodium ion was largely ignored. Once again in retrospect, the reasons for this are obvious. Accurate measurements of sodium were not available, whereas chloride was easily estimated. Important experimental reports were largely dismissed or unnoticed if chloride did not figure in a central role. However the sodium ion was beginning to have protagonists. It was noted that challenging patients with cardiac or renal failure with sodium sulphate produced

oedema of a similar degree as sodium chloride. It was not until 1920 that it was suggested that this was due to the sodium ion itself (Blurn 1920), an hypothesis confirmed by a series of experiments performed by Magnus-Levy (1920), in which patients with renal disease were challenged with a variety of salts of sodium, potassium and calcium to observe oedema-producing abilities. The debate on the possible therapeutic benefit of a low salt diet in hypertension eventually crossed the Atlantic. Meara in his lecture in 1918 at Cornell University clearly distinguished patients with essential hypertension from those with underlying renal disease. For treatment he advised bedrest and suggested it was important "to make most of these patients practically vegetarians", as well as weight reduction for the obese, and a restriction on added salt to food. (The vegetarian diet was a profound step, in view of more recent demonstrations that these groups have lower blood pressures than omnivores). The major protagonist for salt restriction remained Frederick Allen; this was associated with his belief that the chloride ion was the cause of hypertension and that all hypertensive patients had renal disease. In his lengthy report (1922) he cites work on 180 patients, 76 of whom had diabetes or renal failure: the other 104 were divided into two groups by plasma sodium chloride concentrations. There were 109 patients (61%) in whom reduction in blood pressure was observed, but much of this study is open to criticism: first he allowed no control period; second, follow-up was from 14 days to 3 years. No care was taken to incorporate a fixed period of study or to allow for the effects of hospital admission or to control for other dietary changes such as reduction of protein intake. Nevertheless, 19% of the patients attained

a normal blood pressure and 41% obtained some benefit: this study exposed patients to severe dietary restriction of salt (25-30 mmol/day) but blood pressure was lowered in less than half of the sample population. Allen, in fact, explained the failure of other workers to find a beneficial effect of sodium restriction by their inability to keep salt intake low.

Workers quickly tried to reproduce Allen's work; to a large extent the milk diet was now replaced by dietary guidelines with strict attention to detail as advocated by Allen himself. Musser (1920) and Houghton (1922) reported success in 16 out of a total of 17 patients. Again all the work was uncontrolled and is badly reported with no details of duration of therapy. Moreover 25% of the patients had normal diastolic pressures on entry to the diet. A meticulous documentation of one patient on the "Allen" diet was reported by Konikow and Smith (1921). This was reported as a success, although over the 4 months of observation illustrated, I can detect no real alteration in the patient's blood pressure, and certainly it never fell to normal. The authors again urged keeping daily sodium excretion below 1 gm, and they acknowledge a shortfall during their study where the patient clearly failed to adhere to her diet. A further report followed in 1923 with similar successful results (Selman 1923), again with similar drawbacks in its interpretation. One has to be guarded in drawing conclusions from these data; all had trial-design flaws, and patient selection was poor: some had systolic hypertension only, and all the studies were not preceded by a control period of observation. Diets varied in content and most authors at this time (except Allen) found it difficult to keep their patients adhering to an unpalatable diet.

Critics soon reported negative studies: McClester (1922) followed 10 patients on salt-poor and salt-free diets and noted no change in blood pressure. He again gave only systolic pressures, no weights and used many patients with renal failure. Perhaps the most pertinent remarks in this paper are those on how unpalatable the diet was and consequently how little of it his patients ate. In the same year Mosenthal and Short (1923) published their elegant studies: these workers were aware of the minute to minute variations in blood pressure as well as the influences of bedrest and hospitalization. They studied 6 patients and took many blood pressure readings throughout their study and some of the subjects had a control period. Subjects were put on low salt diets and blood pressures did not change. Occasionally the patients received 10 gm NaCl in water without a pressor effect. (Clearly the latter manoeuvre would have no bearing on the patients' sodium balance unless continued regularly for a long time). Nevertheless low salt intake did not change blood pressure. Similar manoeuvres were tried on a patient with severe hypertension reported by Strouse with no effects (1921). Others were equally sceptical: O'Hare and Walker (1923) make this clear from the beginning of their paper. They question the rationale for expecting blood pressure to fall on low salt diets and report findings on 18 patients on low and high salt intakes following control period of between 6 and 18 days. No patients appeared to benefit. Again the authors comment that the very low salt diet was difficult to carry out, monotonous and expensive. They concluded that "salt plays little part in vascular hypertension". Between 1920 and 1940 many workers undertook research into possible beneficial antihypertensive effects of dietary salt restriction.

Not surprisingly opinions were polarised: Hayman (1930) agreed with the reports above, and concluded by saying "I have not been able to recognize any reduction of high blood pressure which I could attribute with confidence to salt restriction nor does a critical review of the literature persuade me of its value". Indeed by the 1930's hypertension reviews were advising against any form of dietary intervention except weight reduction (Adson and Allen 1937). Voices of dissent were still to be heard however: Allen loudly condemned all who failed to demonstrate benefit from his diet, criticising them for not persevering long enough or sufficiently improving the taste of what is undoubtedly a very unpleasant regime. Even at this time some were expounding the virtues of moderately low salt diets as an alternative to extremes of salt deprivation, despite the absence of evidence for efficacy (Blackford and Wilkinson 1932). Blaisdell (1927) tried Allen's diet on hypertensives on an outpatient basis. Some of his patients undoubtedly had renal hypertension and there was no control observation period. Although he did obtain partial reduction of pressure, there were no details of weight changes on this diet which undoubtedly would have occurred as carbohydrate intake was also often reduced. Similar findings were obtained by Vogel (1928); in his study of 212 hypertensives, where 156 patients showed some response to the diet: again no control period was included and some patients had nephrogenic hypertension. Less enthusiastic results were reported in better controlled studies (Chapman and Gibbons 1950) but all series were small and by 1944 interest in salt restriction had waned and few workers were using any form of salt restriction.

At this point it is worthwhile summarising the evidence

on the effects of salt restriction on blood pressure in essential hypertension. The first 40 years of research into this subject produced reports of poorly designed experiments with no control or placebo periods. Many patients clearly had a renal cause for their hypertension. Against this background, however, I think two facts emerged: first, reduction of salt in the diet to extremely low levels, only obtainable by a very unpleasant regime would lower blood pressure in some patients. Of course the attainment of such lower levels of sodium intake required major changes in other dietary components such as fat, carbohydrate and protein. In addition, most experiments failed to consider the hypotensive effects of hospitalization or bedrest. Whether salt restriction was responsible for the success of the diet was not demonstrated but the second fact of interest was that in the experiments where the diet was extremely low in salt, re-introduction of salt raised blood pressure. The most likely reason for any success was probably a reduction in extracellular fluid volume.

The resurgence of interest in salt restriction as treatment for hypertension came with the work of Walter Kempner which began to appear in 1944. Much of the enthusiasm that accompanied his work is attributable to the coverage afforded it by the lay-press. The rationale behind Kempner's dietary regime is difficult to follow: undoubtedly he believed that the kidney was the basic cause of all hypertension and he felt that a normal diet placed an excessive toxic burden on it. Perhaps the best summary of the situation is provided by Pickering (1968): "Kempner ... arrived at this diet as the result of experiments on kidney slices using mental processes that are not accessible to me".

The diet consists of rice, fruit, fruit juices, sugar and adequate amounts of vitamins and iron. It provides 2000 calories daily, 15-20 gm protein, 5 gm fat, and 0.1 to 0.15 gm sodium. Fluids were limited to 700-1000 mls per day. From this outline, it is clear that the diet was not only low in salt, it was also devoid of animal fats and protein. Rice and sugar were the main source of calories. The initial reports were on patients with renal hypertension: however, a later paper described 500 subjects with essential hypertension (Kempner 1948). The duration of therapy was for 4-898 days. There was no effect on blood pressure in 189 patients, of whom 26 were seriously ill and died quickly. Three hundred and eleven showed some improvement defined by a decline of 20 mmHg or more in mean blood pressure, and in 125 of these patients blood pressure fell to 145/95 mmHg or lower. One hundred and forty of the 500 patients had grade III or IV hypertensive retinopathy, and in 88 of these retinal photographs were taken. Papilloedema which was noted in 23 of the 88 disappeared completely in 17, and haemorrhages resolved completely in 39 of 55 patients.

Workers were soon reporting similar experiences with the diet. Grollman et al (1945) treated 6 patients: one had liver cancer and another died from uraemia during the experiment. In the other 4 patients blood pressure was normalised in 2, lowered slightly in 1 and unchanged in the other. Once again these workers also showed the re-introduction of salt raised the pressure - although they challenged the patients with regular diet rather than salt supplementation alone. In Europe the results were similar to those reported by Kempner, with 25% total success. Bryant and Blecha (1947) normalised blood pressure

in 20% of 100 outpatients and improved blood pressure in a further 15%. Again, all this work was poorly controlled and therefore difficult to interpret. Run-in periods varied and many workers set great store by falls in systolic pressure whilst diastolic pressure was often not affected. Once again however, Kempner's work was not uniformly reproducible, and like Allen before him, Kempner became the centre of controversy. Flipse and Flipse (1947) managed to improve blood pressure in 60% of patients but their study was uncontrolled. Little effect on diastolic pressure was shown by Behrendt and Burgess (1947), but successful lowering of the systolic pressure was reported in 7 of 9 mental patients. Results similar to Kempner's were reported by Contralto and Rogers (1948) but here one notes that 38% of the original study population were unable to tolerate the diet. Many studies involved small numbers of patients: Rosenberg and Rosenthal (1948), for example, had only 5 patients on the rice diet with 3 showing some but not complete benefit. Six of Schroeder responders (1949) (9 out of 22) appeared to have Cushing's disease and he was unimpressed with the diet; Chasis et al (1949) saw no effects in 12 patients and Loofbourow et al (1949) saw an antihypertensive effect in 9 out of 47 patients. Similar slight falls in pressure were also reported by Perera and Blood (1947). It is important to note however that experimental design was improving. In 1950 Dole and workers performed careful observations on 6 patients in a metabolic ward; first on a standard diet to acclimatize, then on a rice diet and then with sodium chloride added to the rice diet. The total period of study was 6 months. Five of the patients had significant reduction of mean blood pressure and showed rises in blood pressure when the rice diet

was augmented with sodium (although not to pre-treatment levels). In addition, all lost some weight and some lost a considerable amount (range 2.5-18.5 kg).

In Europe similar studies were undertaken: Bang et al in Denmark (1949) studied 26 patients all still were hypertensive even after 2 weeks in bed ($>180/120$ mmHg). Following this, the patients were placed on a low salt diet (10.51 days), and if the blood pressure fell the diet was supplemented with 77 mmol sodium. To avoid any suggestion effect of the salt addition patients received 'placebo tablets' daily. Of 26 treated, 23 showed a fall in pressure on the diet. The figures are impressive: mean systolic pressure fell 44 mmHg (210 ± 4.9 to 166 ± 5.7) and mean diastolic pressure fell 28 mmHg (123 ± 3.9 to 95 ± 2.9). Rechallenging with salt raised mean systolic pressure 22 mmHg and mean diastolic pressures 6 mmHg. The British experiment was performed at the request of the Medical Research Council in 1950 (MRC 1950). Thirty-six patients with a diastolic blood pressure equal to or greater than 120 mmHg were studied. This report notes that the diet was unappetising and monotonous, necessitating the withdrawal of some patients: many could not wait for the desired period of study (6 weeks) to expire. Blood pressure fell in most subjects: in 35 of 36 patients the mean fall was 55/26 mmHg. There was a rise in pressure quickly after changing to an ordinary low sodium diet (17-51 mmol sodium per day). All patients lost weight and those with oedema showed rapid improvement. The report concludes that the Kempner diet can be expected to produce a considerable fall in blood pressure in about 70% of patients with either renal or essential hypertension, provided that they adhere strictly to the regime. However,

the blandness of the diet made it difficult for patients to comply over long periods and even the return of a small amount of salt caused the pressure to rise again. The hypotensive effect of the diet was associated with reduction in plasma and extracellular fluid volumes (Murphy 1954, Watkin et al 1950). The rapid return of blood pressure to high levels with the re-introduction of salt was associated with weight gain and thus probable increases in plasma and extracellular fluid volumes (Hatch et al 1954). The failure of salt restriction to lower blood pressure in some patients with hypertension or essential hypertension may reflect compensatory activity of the renin-angiotensin system which maintains blood pressure in the face of a reduced blood volume.

With the widespread introduction of diuretics into clinical practice around this time enthusiasm for the pursuance of these dietetic regimes waned. Nevertheless the work I have described above is invariably quoted as being evidence that amelioration of dietary salt intake will lower blood pressure. More alarming is the inference from these that more modest reductions in salt intake might also be useful. Primarily, apart from the studies by Dole, Bang and the MRC, little of the experimental work on dietary manipulations and blood pressure during the first half of this century was controlled. What does seem clear is that Kempner, and before him Allen and Sherill demonstrated drops in blood pressure on their low sodium diets. In all the experiments patients lost weight, a factor known to lower blood pressure independently even though here it implies loss of fluid. Most studies were uncontrolled; (workers have demonstrated the power of suggestion in the treatment of hypertension). Undeniably

however, some workers, especially Kempner obtained extremely good results with his diet. Pickering (Simpson 1979) was sceptical of the regime; he states that he was mystified as to why his patients would not adhere to the diet for very long whereas Kempner's did. Upon visiting Kempner to find out why, Pickering found Kempner to be a fervent believer in his diet but also discovered him to be a "Prussian and also a bully and that he regimented his patients". Indeed some workers attribute Kempner's success to his sincere belief and rigid discipline at a time when most physicians could offer little or nothing for patients with severe hypertension. Whilst undoubtedly benefitting many in some workers' hands, the rice diet could also be dangerous in provoking uraemia, and it is worth noting that some patients were made worse and died whilst being treated with it. The other point of interest is whether the sodium ion was the only component in the diet linked to the reductions of blood pressure seen with such extreme diets. Both Allen and Kempner used diets where most of the calories were obtained from milk, rice or sugar. These diets are devoid of most animal fat and protein, and serum cholesterol was invariably shown to fall. Nearly 60 years ago hypertension was found to be extremely rare amongst Greenland Eskimos who consume a large amount of animal protein and fat (Thomas 1927); moreover, Saile (1930), and Raab and Friedman (1936) reported lower pressures in vegetarian monks compared to omnivores. A recent experiment performed by Sacks et al (1981), challenged strict vegetarians with meat and demonstrated a rise in systolic pressure and pulse rate, which reverted to normal upon withdrawal. Sodium excretion remained constant throughout the study. Other workers have shown that a change to a strict vegetarian diet

will lower pressure (Rouse et al 1983); Kempner's diet produced dramatic changes in fat and protein content as well as salt. This said, the most likely factor is the loss of fluid volume provoked by the reduced sodium intake. Herein lies a problem for the advocates of moderate salt restriction. The addition of salt to a level corresponding to moderate salt restriction still raised pressure. Of course, some workers merely restarted patients' normal diet. But Bang et al used tablets (1949) and the MRC study (1950) was equally careful choosing to re-introduce salt alone into the diet. It is likely that the dietetic influences on blood pressure were multifactorial. Thus, it may be not only the reduction of salt in Kempner's diet that is important but also the changes in fat and protein. Two pieces of evidence support this: the first is that the re-introduction of salt raised blood pressure in patients on Kempner's diet but not to the original levels, unless a normal diet was employed. Second, some sophisticated studies in the rat demonstrated that high levels of saturated fat irrespective of dietary salt can induce increases in pressure, and that both fat and salt can interact to influence blood pressure (Smith-Barbaro et al 1980). Certainly it is fundamentally wrong to single out salt as the prime dietary suspect in essential hypertension using as evidence the extremes of salt intake described above.

With the introduction of a powerful array of antihypertensive drugs, interest diminished in stringent unpleasant dietary control of blood pressure. The salt controversy was continued in a different sphere with a variety of epidemiological studies of primitive races with apparently low salt intakes and a low incidence of hypertension. These are discussed elsewhere. Stimulated

by such research, and by the powerful argument that an even small downward shift of a whole population's blood pressure might have a greater impact on cardiovascular morbidity than merely lowering the pressure to normal in those with definitely raised blood pressure, attention was focussed on possible modifications of lifestyle of Western Man. Reports began to appear involving moderate salt restriction in hypertensives. The first of these came from Belgium (Parijs et al 1973). This work was performed on 17 hypertensive subjects who were placed on normal diet, normal diet plus thiazide diuretic, moderately low salt diet and moderately low salt plus thiazide diuretics. The period was 4 weeks on each regime. Blood pressures were measured by the patients at home, and in the outpatient department. The authors set great store by falls in the home blood pressure measurements on diuretics, low salt diet and low salt diet and diuretic regimes: However there was no hypotensive effect demonstrated on the low salt period when the outpatient blood pressure was measured. In fact, supine diastolic pressure actually rose when urine sodium excretion fell from 191 mmol/24 hours to 92.8 mmol/24 hours on modest sodium restriction. The standard deviations were 61.2 mmol/24 hours on regular diet and 41.8 on low salt diet. Evidently the individual changes in intake varied from enormous to non-existent. It is difficult to infer a great deal from this work: all subjects had been receiving antihypertensive treatment until 2-4 weeks before entering the study. Some had been receiving low salt diets before entering the trial. There was the inevitable weight loss on the low salt intake and although small it is important to note that any falls in pressure noted on the home measurements were also small. The

dietary regimes were not randomised and both low salt periods occurred together. Overall the information obtained must be regarded as unhelpful.

Two years later saw the salt debate break out again in the Medical Journal of Australia, with Dr Kincaid-Smith advocating a "no evidence" standpoint being attacked by Drs Snibert and Morgan. Morgan had been conducting studies of the effects of various treatments on blood pressure since 1973. It is clear from his writings that he has been and remains an ardent believer in the evils of sodium chloride. In 1975, he reported the effects of reducing the sodium intake of 12 hypertensive men (Morgan et al 1975). The 24 hour sodium excretion fell from a mean of 205 mmol to 120 mmol/day and mean blood pressure was significantly reduced. Most were already on drug therapy and the usual criticisms apply; there is no way of saying that sodium restriction was the mechanism of the further fall in pressure or whether it was the effects of close regular outpatient supervision for example. This report was followed by a further study (Morgan et al 1978). Here, Morgan treated 31 men with a diet designed to moderately lower sodium intake for 2 years: others received no treatment or drug therapy. The mean urinary sodium excretion fell 38 mmol/24 hours implying some patients almost completely failed to change their diet, a point acknowledged in the discussion. Nevertheless, mean standing blood pressure and supine diastolic pressure fell significantly. It is hard not to be sceptical of these data. Blood pressures were only measured to Korotkoff sound phase 4; there are no details of any weight changes, and the urine collections were sporadic and single specimens only. Nevertheless, the authors conclude that "if a person presents

with a diastolic blood pressure between 90 and 105 mmHg or has a family history of hypertension, he should be advised to reduce his sodium intake to 70 mmol/day."

The best study of moderate salt restriction in hypertension is that of MacGregor et al (1982). He reduced salt intake of 19 patients to 80 mm/day and then in a double-blind fashion gave 4 weeks Slow Sodium or 4 weeks placebo. Mean blood pressure fell significantly on the placebo period and rose on rechallenge with Slow Sodium. This is a well designed and meticulously conducted trial. Weights did fall slightly on the low salt diet as did potassium excretion. Overall mean blood pressure fell in 13 subjects, was unchanged in 3 and rose in 3. The experiment remains the best evidence in favour of moderate salt restriction being useful in hypertension, and furthermore in view of the pressor effect of Slow Sodium demonstrated that excessive salt may be responsible for rises in pressure in essential hypertension. One concern is the short term period of experimentation: admittedly the authors do say that 9 patients have continued the diet for 6-9 months with continued benefit. A prolonged period of observation would be relevant when recommending life-long salt restriction for lowering blood pressure. Subsequent studies have not been so successful; Puska et al (1983) reduced sodium excretion from 192 to 77 mmol/24 hours with little effect on blood pressure. Unfortunately blood pressure showed a progressive fall in the first part of the study owing to too short a run-in period. Watt et al (1983) effectively repeated MacGregor's study in a general practice with 20 subjects restricting their sodium intake. Hypertension was less marked than in MacGregor's study, blood pressure was not affected, and once again compliance

was found to be difficult to achieve. Two patients did not complete the trial being unable to tolerate the diet and 5 patients blatantly did not restrict their sodium intake or take the Slow Sodium tablets. A further negative study followed from Silman et al (1983), who again demonstrated difficulty in maintaining dietary compliance with little fall in sodium excretion and no change in pressure compared with the control group. This study concluded that any reduction in blood pressure seen with a low salt diet was no different from that seen with constant supervision and weight loss. This theme was pursued by Fagerberg et al (1984): two groups of obese men were given a weight-reducing diet and their salt intake kept constant on a diet designed to give a moderate restriction of sodium intake and calories. Blood pressure fell on energy restriction with normal sodium intake but not significantly. However, blood pressure did fall significantly on the low salt low calorie intake. The diet periods were not randomised, nor were the groups crossed over the receive both diets. Alcohol intake fell more in the group on low salt low calorie diet. The case is far from proved that decreasing salt intake was the factor that made the low salt low calorie diet lower blood pressure.

A more recent study of moderate sodium restriction came from New Zealand and was negative. Richards et al (1984) placed hypertensives on 4 weeks of reduced salt intake and monitored blood pressure on an outpatient basis as well as during a period of 24 hours intra-arterially. The small falls in pressure observed did not attain statistical significance. This study has been criticised for failing to demonstrate changes in renin and angiotensin II when subjects were on the lower salt intake, and that

some of the subjects clearly were not hypertensive. Nevertheless, a clear cut lowering of blood pressure was not seen. Similar findings have now been reported in young modestly hypertensive subjects (Grobbee 1985).

After 80 years of experiments designed to test whether salt restriction can lower blood pressure we are little better off; whilst some diets used to restrict salt intake severely are effective by reducing fluid volume, the more recent experiments with moderate sodium restriction are for the most part negative. Where moderate sodium restriction is not associated with evidence of fluid loss, no effect is seen, and many subjects compensate by stimulating their renin-angiotensin axis. Again this explains the lack of success in lowering the blood pressure of normotensive subjects with such diets. Positive studies (such as MacGregor's) deal with more marked degrees of hypertension and in such individuals, the renin response to salt restriction is often more severely impaired. The case implicating salt excess as causal in the genesis of essential hypertension is far from proved on the evidence above, and furthermore the ameliorating effect of salt on blood pressure is far from clear cut until hypertensive individuals reduce their sodium intake to very low levels.

Cross Cultural Studies of Salt Intake and Blood Pressure

The protagonists of the view that a high salt intake is the major contributor to elevated blood pressure, cite the fact that primitive man ate a diet low in salt and further that hypertension is rare amongst primitive cultures scattered around the world who consume very little salt. On the face of it from the evidence cited, this would appear to be a powerful argument: however detailed examination of the evidence reveals that this

is far from proved.

In this section the evidence for this belief is scrutinised and analysed. The major problem with the argument is that whilst no elevation of blood pressure appears to occur in most of these primitive peoples, the studies themselves are for the most very poor in data to support the contention that absence of salt is responsible.

In a sense, the main drawback when trying to make the salt hypothesis believable, is the fact that measurements of salt intake were not the prime objectives in these studies. Observations on blood pressure and salt were often incidental. Nevertheless blood pressure in many of these investigations did not seem to show the same rise with age as was observed in Western cultures. The most comprehensive studies come from New Guinea; Sinnett and Whyte (1973 a,b) meticulously established that cardiovascular disease and, in particular, hypertension were virtually unknown in several regions of the highlands. The main dietary constituent is the sweet potato, which is supplemented with rice, some carbohydrates from sugar cane and a little pork or wild fowl. The diet, therefore, is low in salt, but there are also differences in fat and protein content from Western Man. When available salt is added, it is often in the form of plant ash and therefore rich in potassium. My reading of the studies reported from this area suggests that the collection of hard data on sodium intake by these tribes leaves something to be desired. Sinnett and Whyte collected 24 hour urine saves from 272 subjects; however, it appears they only had one save from each subject making interpretation difficult (Watt and Foy 1982). No correlations were made for body mass or creatinine. Nevertheless, mean 24 hour sodium excretion

was 13.7 mmol for females (n=135) and 6.4 mmol for males (n=138). These figures agree with those obtained in other reports (Whyte 1958): that the salt ingestion of these people is extremely low seems irrefutable. Nevertheless, the correlations with blood pressure and sodium excretion were non-existent (-0.085 systolic for males, and -0.04 for females: -0.13 diastolic for males, and 0.088 for females). This would be expected bearing in mind that only single collections were made and the range of sodium intake is apparently very narrow. Other dietary constituents were also measured and animal fat intake was also low with a high protein consumption. Seventy-three percent of males and twenty percent of females smoked, but few inhaled. Eosinophilia in the peripheral bloodstream was a finding in 20% of the natives but overt parasitism was very rare. Serum cholesterol was much lower by European and American standards. Overall, it seems clear that these subjects differed markedly in many ways compared with their Western counterparts apart from just their salt intake. Most subjects were lean but in this study malnutrition was not evident: other studies however, put the prevalence of nutritional problems higher: Marasmus and Kwashiorkor are reported and there are areas with a high incidence of xerophthalmia, and rickets (Oomen 1961). In the high valleys of the interior there is an endemicity of goitre which is often serious. Malnutrition in some areas was the main cause of death in patients admitted to hospital (Ivinskis et al 1956). Clearly, the dietary patterns vary from area to area; along the coast and in the interior these patterns depend on the native crop, which is the sagopalm. In the foothills, it is on taro yams and bananas. Above 2000 metres, it is the sweet potato. All contribute to what is a

low sodium diet and much of it is supplemented by rice. The New Guineans on the coast eat shrimps, shellfish and fish: (clearly their salt intake will be higher than the highland dwellers). They rely on sweet potatoes and leafy vegetables, and in all these diets potassium intake is high. The picture is further confounded by the ever increasing contact with Western society in recent years, with modifications to diet being an inevitable consequence. The dietary constituents alter with regard to fat intake as well as the amount of salt contained: moreover, blood pressure has also been shown to rise (Ivinskis et al 1956; Macfarlane et al 1982). However, it appears that blood pressure fails to rise with age. Stanhope (1968) produced a poor two page report in 1968 when he measured the blood pressure of 556 of 917 people of the Tinam-Aigram people and confirmed this. However, there are no other data on diet or salt intake and there were few people over the age of 50 years in the study. Nevertheless, reports from other workers which are more detailed corroborate the findings. Interestingly, Barnes (1965) who studied the Lower Bomai people comments that "fat contributes less than 5% of the daily caloric intake and is almost exclusively unsaturated vegetable fat". Once again we have further evidence of a change in a major dietary constituent other than salt, which is known to alter pressure. Barnes found no case of hypertension in 161 adult Lower Bomai people, with no overall trend for pressure to rise with age (although the males did show a trend to rise, the females did not). Maddocks and Ravin (1965 and 1967) emphasised salt as a factor, although the reason for selecting this rather than other possibly relevant variables is not clear. They reported a fall in blood pressure with age

in Chimbu natives, although the explanation may well be due to the small numbers over 60 years whose pressure he measured. Moreover he states that the life expectancy of these natives is "probably less than 40 years". Whilst conceding major differences in the fat and protein constituents of the diet, he concentrates on the natives low salt intake as being responsible for his findings. He concluded by contending that the pattern of blood pressure seen in these natives might reflect that seen in Stone-Age man.

Elsewhere in the Pacific, other populations have been studied: Lot Page and co-workers (1974) analysed cardiovascular risk factors in six groups of people in the Solomon Islands. His study was set-up as a result of observations that acculturation seemed to lead to the elevation of blood pressure and the appearances of risk factors in primitive tribes. Six melanesian tribes were investigated, all had low levels of acculturation and differed in their degree of exposure to Western habits. The results of the study suggested once again that blood pressure failed to rise with age in the most unexposed and unacculturated tribes. With increased exposure to Western influences serum cholesterol rose and blood pressure showed the Western tendency to an age-related rise; this appears to be most noticeable amongst females. Once again these tribes showed an abundance of intestinal parasites and slight anaemia. Cholesterol levels were higher in the three more acculturated tribes compared to the three who were less so; Page attributes this rise in cholesterol to the introduction of animal fat in tinned meat and fish. In his discussion, the authors suggest several factors which might be elevating the pressure: whilst they acknowledge that social and family roles

were unaltered, entry into a cash economy and wage earning employment altered the males. However, these showed less pressure elevation, and Page points out that the most likely factor to incriminate was the dietary changes. He felt that the most crucial change was in salt intake - a factor only investigated by recall studies. No importance is attached to the fat and protein changes. In reviewing Page's work Simpson (1978) felt that the divisions between the six tribes with regard to degrees of acculturation were arbitrary. The tribe with the highest pressures cooked in sea-water but these people also lived in cramped conditions. There is probably some truth in this viewpoint which emphasises the difficulty in identifying confounding variables. However, Page countered this in his discussion, noting that many men and women left the islands during the day to engage in gardening and fishing in the surrounding uncrowded lagoon and mainland. The authors also point out that similar crowding was reported in Botswana and blood pressure remained low throughout life. Salt was by no means the only variable in the tribes' lifestyles and measurements of salt intake in this study are sadly lacking. Once again salt seems to have been implicated rather prematurely. Indeed as in most of the studies in this chapter this investigation was not designed to examine the putative role of salt in the genesis of hypertension; rather to look at whether blood pressure showed an age-related rise in unacculturated tribes. Data on why it did not are scanty and the implications of such data that exist are questionable. What is conclusive from these experiments is that blood pressure does not rise in some Pacific races. In addition to New Guinea and the Solomon Islands, Maddocks (1961) found the same phenomenon in Melanesians. Moreover he surmised that the differences between these island dwellers

and Western people might be due to climate and added that "It has not proved possible so far to implicate diet, salt intake or smoking habits". In the same geographical location, another study reported similar findings to Page: Prior et al (1968) found higher blood pressures in the Raratonga tribe compared to the PukaPuka people. The Raratonga were more acculturated and had higher salt excretion than the PukaPuka. This was due to an increased ingestion of corned beef, tinned fish and salted coconut sauce. At the time of study the Raratongans had been under Western influence for 10 years. Fat and protein changes in the diet were not considered, blood pressures were only measured to phase IV and much of the urine sodium data was based on casual spot urine collections or on incomplete 48 hour saves. No attempt was made to correlate sodium excretion and blood pressure levels. Other studies have also confirmed environmental influences to be of great importance in raising pressure: Cruze-Coke et al (1964) showed that blood pressure of Easter Islanders who migrated to the mainland of South America rose irregardless of age. Prior and co-workers (1974) examined Polynesians from Tokelau; he subdivided his populations into those that subsequently migrated to New Zealand and those that remained on the Tokelau atol. The male pre-migrants were taller and heavier and had slightly higher blood pressures than the males who remained on the island. However, little was found to be significantly different. Interestingly, Prior attributed the changes to the selective processes whereby the younger more athletic males indulged in migration. Certainly the sodium consumption did not differ significantly in the two groups.

From the studies of Pacific races several things become

clear: first, essential hypertension and cardiovascular disease are virtually absent, and secondly the diet is radically different from Western Man. The data on salt intake are the weakest aspect of the reports and yet have achieved the most prominence. I have included only those studies in this geographical locale that mention sodium intake; many others confirm the trend above, but data on salt are completely absent. Exposure to social and economic change appears to influence blood pressure patterns in these communities, but it would seem unjustified to select out salt as the root cause on the basis of the evidence cited.

A similar phenomenon of low pressure communities is to be found on other continents. In addition, most of these reports contain no firm data on the salt content of the diet. The often cited study of Kalahari Bushmen (Kaminer and Lutz 1960) indeed reveals no blood pressure rise with age but only a brief outline of a predominantly vegetarian diet (known to be associated with lower pressure (Sacks et al 1974)). Africa also provides evidence of life changes again raising blood pressure with Shaper's study of Samburu nomads entering the army (Shaper et al 1969). Again a diet radically altered in protein and fat content. These warriors had a sodium intake calculated to be similar to Western Man before entering the army. After entering the armed services weight rose as a result of increased caloric intake as did blood pressure. Truswell and co-workers (1972) reported on the failure of blood pressure to rise with age in male Kung Bushmen in Botswana. The females did show a slight rise in systolic pressure - a feature reported in other tribes above. Their staple diet is based on the nut of the Mongongo tree and is rich in fat and protein. In his review of these peoples, Denton (1982) cites

the sodium excretion of these Bushmen to be very low at 2 gm per day. However, detailed study of the paper by Truswell reveals yet again virtually no hard data on sodium intake. Twenty-four hour urine specimens were collected from six Bushmen in 1967 and four of the same Bushmen in 1968. No volumes are given for the urine collections and the data are presented as not susceptible to scientific interpretation. In Sever's study of the Xhosa blacks in South Africa (Sever et al 1980) urban people had higher pressures than rural blacks. The urban Xhosa were heavier and excreted more sodium (on spot urine analyses) but there was no within-population correlation of blood pressure and sodium excretion. It is also of interest that the lower sodium intake in the rural Xhosa was approximately of the same order as is observed in high salt Western cultures.

In 1978 Page et al published a small report of his studies in Iran. Jere is a nomadic tribe which feeds on bread, rice and dairy products. Blood pressure was found to rise with age and correlate with sodium excretion in males. The sodium excretion was measured on overnight urine collections, with diet assessed on recall from the previous day. One thing quickly becomes apparent from this paper, namely the variability in dietary foodstuffs taken from day to day. For example, many times the nomads ate just bread one day and rice another. One timed urine save must be viewed with some scepticism as an index of sodium intake, but did approximate to that seen in Western Man. Similarly, Schneckloth and co-workers (1962) found higher blood pressures among the negroes of the crowded sugar plantations of St. Kitts. Spot urine tests and dietary analyses led him to believe these people had a salt intake equivalent to whites in the Bahamas

and USA. In addition, an American nutritional survey of Buddhist farmers in Thailand found a dietary salt intake of 340 mmol sodium per day (Thailand 1969) but very little elevated blood pressure. No urine collections were carried out so information on sodium intake is not available.

Low pressure tribes are also found in South America, but it was not until 1975 that any quantification of their salt intake was attempted. Then Oliver et al (1975) reported their study on the fierce Yanomamo Indians of North Brazil and South Venezuela. In his introduction Oliver notes that few studies of primitive tribes had been studied so close to their first contact with acculturation, and having found no elevation of blood pressure with age he decided to examine sodium intake of these Indians. The staple diet is based on cooking banana with irregular additions of game, fish, insects and wild vegetables. The authors managed to obtain 24 hour urine saves from 44 subjects, although state that "the collections were complicated by the rather difficult conditions under which the field work was conducted as well as the free and unfettered nature of the Yanomamo psyche, which found it difficult to take seriously the concept of a 24 hour urine". Only just over half the collections could be regarded as anywhere near complete, although the criterion used (urine creatinine 500 mg) was arbitrary. the authors reported a reversal of the Na^+/K^+ ratio with mean sodium excretion of 1.02 mmol/24 hours and potassium excretion of 152 mmol/24 hours. Despite the shortcomings of the measurements made, the conclusion that the salt intake of these people was low seems inescapable. However, Oliver felt that it would be simplistic to ascribe the failure of blood pressure to rise with age to the salt poor

diet. He points out that these Indians are thin, do not gain weight as they age, and lead an extremely aggressive lifestyle. In addition, they have been the subject of further intense observations which throws doubt on some of the initial reports of their customary diet. Chagnon and Hames (1979) investigated the possibility that the aggression of these Indians might be due to protein deficiency. Here they found abundant intakes of animal protein with an average of 50 gm of protein per head of population per day provided by the hunting males. Fishing provided 25 gm protein per head per day. These figures do not include the protein derived from caterpillars, grubs, termites, ants and bee larvae, plus crabs, frogs and tadpoles. Chagnon and Hames therefore estimated the protein intake of the Yanomamo to be higher than some Western nations, and some 250% higher than the highest estimated requirement. They also noted that these Indians are among the world's smallest people and thus they eat nearly twice the amount of protein recommended by the American National Research Council. This also makes the sodium intake studies of Oliver difficult to assess: Clearly the sodium intake of the Yanomamo cannot be as low as was originally perceived, and allowing for losses in sweat, is probably somewhat higher although it may not be near that of Western Man. It also seems likely that it is widely variable from day to day.

There are fewer examples of high salt cultures. However in some parts of Japan the intake of salt is enormous. Data are however inadequate. What appears to be clear is that on the North Island the salt intake is between 2 and 3 times higher than the average intake in the UK (Takahashi et al 1957). Salt is taken in soya sauce and pickles; and the prevalence of hyperten-

sive complications in these people is high. However, the incidence of such hypertensive problems is far lower in other Japanese areas where the salt intake is only 50-70 mmol lower (Sasaki 1962). Moreover other dietary constituents change with the latter populations eating less fish and rice.

Overall therefore, it would appear that blood pressure shows a tendency to rise with age in the majority of populations studied, both acculturated and unacculturated; in a small number of primitive peoples with only scanty exposure to modern civilisation, this age-related upward trend appears to be absent. The introduction of such radical changes in lifestyle seems to initiate a rise in pressure: the factor or factors which cause this are unknown. Data on the salt intake of low pressure communities are grossly inadequate and in some studies have to be reconstituted from minimal dietary evidence. Many primitive tribes had unpredictable daily food intakes and thus one urine save is meaningless. Whilst the salt intake in these communities is probably lower than the current national UK average, their diets are dramatically different in fat and protein. Similarly, exposure to civilisation raises these parameters. These observations are of more than passing interest: for example, Sacks et al (1974) studied blood pressure and dietary habits of 210 men and women in a Boston macrobiotic commune. He found that blood pressure was lower and the age-related increase significantly less than that usually found in Western populations. It seems reasonable to postulate that daily dietary intake of nutrients can influence cell function to some degree, although the stress of competition in jobs, wage-earning and lifestyle may also clearly contribute. Here the views of Henry and Cassel (1964) are of relevance because

they feel that obesity and dietary factors may not be as significant in explaining variations in blood pressure levels in different populations as perhaps is the organism's perception of events in the social environment. Difficulties of adaptation, where there is what Henry terms "status ambiguity" lead to years of repeated arousals of vascular, autonomic and hormonal function due to man's perception of various events as threatening. These it is claimed can lead to progressive disturbances which eventually raise blood pressure to a level set higher than normal. These opinions are supported by an array of evidence both circumstantial and experimental in Henry's comprehensive review. Certainly the social upheaval in primitive peoples when brought into contact with modern civilisation must be dramatic: placed in this context, to single out dietary salt intake as the most important pressor agent seems far-fetched. Dahl's (1960) plot of salt intake against percentage of population found hypertensive, suffers from similar problems in as much as the firm data on actual salt intake are sparse with reliance placed on dietary recall. Pickering (Simpson 1979) went further in criticising this work. He correctly points out that nearly all the points lay absolutely on the line with only 2 or 3 points just a little out. However, he then continues; "How on earth do you measure the percentage of hypertension in the population when you do not know what is the dividing line between 'hypertension' and normal blood pressure?". Moreover there appears to be no controlling for age in these data and although this graph is reproduced ubiquitously the information contained in it is poor. Glibermann's (1973) work suffers from similar drawbacks with poor salt intake data and no correction for age or weight. Neither study improves

our knowledge or resolves the issue. Even where 24 hour collections have been carried out, the statistical power of a single measurement is very low: it has been estimated that up to 7 collections are required (Liu et al 1979). This may equally explain the failure to find a correlation between blood pressure and salt intake in single cultures.

The final consideration to this aspect of the argument must be the convention that the diet of these primitive tribes described above reflects the habits of the primitive ancestors of Western Man. The inference is that hypertension is a disease of modern living which has been inflicted on the population by the excessive salting of food that occurs today. Because we have been only able to measure blood pressure for 100 years, the case is impossible to dismiss. However, a closer examination of our ancestors' nutrition yields interesting information. For example, one of the largest contributions to our daily salt intake is bread. The amount of salt in bread in the UK rose between 1760 and 1936 and in the years since then it has fallen dramatically (Hémardinquer 1970). In fact, we are now eating a much smaller amount of salt than 400 years ago. In 1565, Edward Baeshe was contracted to provision English Navy ships at a price of 5d per sailor per day (Hémardinquer 1970). Here salting of food was commonplace and for voyages preserved food was of extreme importance. Salting of food was commonplace at this time and it is probable that non-naval people consumed as much as members of the armed forces. A simple calculation is sufficient to tell us that just the daily meat ration contained 213 mmol sodium without taking any beer (of which 4.6 litres was available per man), butter or dried fish. However the situation

seems not to have changed radically 300 years later. The diet of armed forces - less reliant on salt for preservation in view of the reduction in voyage times - still contained as much or even more salt than in 1565 (Hé mardinquer 1970). With life expectancy curtailed by other diseases to a mean age at which hypertension would only be about to increase morbidity, to extrapolate further back in time seems futile.

In conclusion, the evidence that it is the absence of salt in the diets of primitive tribes which keeps blood pressure low is poor and the case is over-stated. Changes in acculturation and other nutrients may be equally or more important. To criticise the studies on the grounds of poor blood pressure measurement, failure to correct for arm circumference or age factors seems unnecessary when the data on salt are so inadequate. These anthropological studies are interesting but unhelpful in what is such a multifactorial disease.

Sodium Intake and Blood Pressure Studies in Acculturated Man

For many years workers have tried to correlate blood pressure levels to salt intake in populations. Such experiments are fraught with difficulty which stem from the acknowledged variations in the parameters being studied. Measurements of blood pressure are usually collated from casual readings although it is accepted that blood pressure varies from minute to minute. Thus to try and find an association between one momentary blood pressure and a subject's sodium excretion begins as an ambitious exercise. The problem is compounded by the difficulty in accurately assessing sodium excretion (Liu et al 1979). Many studies quoted above have suffered from such flaws with dietary recall analyses being particularly notorious. One urine save for 24 hour sodium excretion

may be incomplete, and spot urine measurements are just as inaccurate. It is not surprising therefore that most studies are negative.

Dahl and Love (1953) based their study on dietary recall and then arbitrarily divided estimated salt intake into low, average and high, finding hypertension distributed in the average and high groups, although there was only 50 mmol/24 hr difference between average and high levels of excretion. However this highly significant study is an exception rather than the rule. When Miall repeated this experiment in the RhonddaFach (1959) no such association was found. Yamori et al (1981) reported a correlation between systolic blood pressure and sodium:potassium ratio in 111 subjects in Japan. Close examination of this however leaves interpretation open to question. The nutritional survey Yamori undertook was a comparison between two farming and fishing villages, where stroke incidence was high in the farming village and low in the fishing community. Only one urine save was collected and sodium excretion was similar in both communities, and only by using multiple regression analysis were sodium:potassium excretion ratios correlated with systolic pressure. No correlation was observed with sodium excretion and blood pressure alone. Khaw (1983) examined the casual urine samples of 93 London factory workers and reported significant correlation between systolic blood pressure and sodium/creatinine and sodium/potassium ratios. Khaw's claim that a 10 mmol increase in 24 hour sodium output accounted for a 3 mmHg rise in systolic pressure is remarkable and requires further investigation. Similar but less significant correlations were subsequently reported by Strazzullo et al (1983) in Neopolitan men. However, other studies have been negative: Bulpitt et al (1976) found no association between sodium output

and blood pressure in 689 general practice patients. Similarly Omvik found no association in Norwegian men, and Simpson et al (1978) performed negative studies in New Zealand.

One possible explanation for the frequent lack of a possible association between salt intake and blood pressure is that the range of sodium intake is not sufficient to show up any effect on blood pressure. However this argument is refuted by a study by Ljangman et al (1981). These workers describe the blood pressures of 120 Swedish men distributed across a large range and again failed to establish a significant correlation. Large scale studies such as the first National Health and Nutrition Examination Survey (NHANES 1) were based on dietary recall data. Using this information Harlan et al (1984) examined a sub-sample of 3854 adults and found no consistent relationship between the salt content of food consumed and blood pressure. Similar findings from 3500 people from Connecticut were reported by Holden et al (1983). Kesteloot (1984) reported on a major survey of 9321 men and 421 women in the Belgian army and once more there was no effect of sodium output on blood pressure. So large was this study that it had the power to detect minor differences in blood pressure and yet was negative in outcome.

The overall conclusion must be that studies attempting to associate sodium intake and blood pressure are negative. However the difficulty in adequately measuring the two parameters concerned still leaves interpretation of such studies open to debate (Watt and Foy 1982). Nevertheless, whatever methods used, most workers have been unable to associate salt intake and individual blood pressure levels.

If the excessive intake of salt is the factor responsible

for raising pressure in hypertension, challenging human subjects or animals with a high salt load should demonstrate a pressor effect. The next section deals with the evidence cited in favour of this premise.

Animal Studies

The first experiments in this field were performed on chickens. In 1947, Krakower and Heino (1947) published data demonstrating a rise in blood pressure on a high salt diet. This was confirmed by Lenel et al (1948) who showed a rise of 51/37 mmHg in 8 chickens fed 9% and 1.2% saline. As in many such experiments described below the birds were presented with enormous amounts of salt: indeed, in the latter experiments this was all the birds drank for 121 days apart from two control periods when tap water was introduced. I raise this criticism not solely to detract from a positive result but to focus attention on the health of poultry involved. Both groups of chickens were extremely ill during the study. Many had profuse bloodstained diarrhoea and some birds died before the study was completed. The study by Lenel is statistically inaccurate with data from 5 chickens at the end of the study compared with those from the 8 which started it. Some birds in both studies became hypertensive and some did not; the response was by no means uniform. (Lenel's birds were all hypertensive at the outset, a phenomenon he attributed to their "high normal" body temperature of 42 °C). Most birds showed evidence of fluid overload and the role of salt per se as the pressor agent apart from causing volume expansion cannot be assumed. Other species such as the rabbit were also studied by Fukuda (1951). A slight rise in blood pressure was observed as the result of 5 gm of salt daily for several months. The rise began approximately 4 weeks after the initiation of the high salt intake and persisted for 8 months. When

the high salt intake was discontinued the pressure gradually fell over 4 months. However, only the systolic blood pressure in the central artery of the ear was measured and the results could not be confirmed by later studies (Goldblatt 1969).

Experiments in the rat initially demonstrated negative results (Grollman et al 1940), but Sapirstein et al (1958) showed a pressor effect of saline-fed animals. However, some animals died during the study; all saline-fed rats were lighter and the blood pressure response varied from animal to animal, and again some animals were rendered ill with diarrhoea from the saline purge.

Meneely and his co-workers (1953) were the first to perform studies designed to examine salt ingestion itself. They reported on the effects of 7 different levels of sodium chloride on the growth and blood pressure of male albino Sprague-Dawley rats. The experiment was designed to cover the life span of the rat and the diets were meticulously designed with the sodium chloride level varying from 0.01% (low NaCl) to 9.8% (high NaCl). At 14 months after starting the diet 50 out of the 193 animals (31%) that began the experiment had died or had been sacrificed. Blood pressure rose in all groups (including the low salt group), but most of all in the high salt intake groups. These animals showed post-mortem evidence of oedema and fluid retention. Once again the animals had received enormous amounts of salt and were chronically unwell; data on those that died before the end of the study are not given. Nevertheless, like the experiments above the workers showed a rise in blood pressure with adding salt to the diet, although there was considerable scatter in the groups with some animals on a high salt intake remaining normotensive and blood pressure rising in some of those on the low salt diet. Ball and Meneely (1957) concluded in a further study

that "excessive salt was rough on rats". Here, they also reported an increased mortality in the animals on high salt intake after 17 months on the diet. Similar results were also reported by Battabee et al (1979) who fed Sprague-Dawley rats high sodium diets and detected a pressor response and increased urinary excretion of noradrenaline. However these workers selected the animals who were studied for urinary catecholamine studies which were performed in metabolic cages known to stress animals and this data must be regarded as questionable.

All the studies described above were performed on young offspring; Grollman and Grollman (1962) however, demonstrated that the provision of a high salt intake (2% saline) from 7 days before conception or from the 5th day of pregnancy produced a rise in pressure in a significant number of the offspring of Piebald rats. In their discussion, the authors comment that the results demonstrated that environmental factors affecting the foetus before birth "might readily account for many instances of spontaneously occurring essential hypertension"

A variety of criticisms may be made concerning all these studies. Many were levelled by Simpson (1979); whilst conceding that a heavy salt intake can raise blood pressure, the amounts used in the studies described above were "simply enormous". Some of the rats were given a salt intake equivalent of 9520 mmol of sodium daily in man. Vogel (1966) used doses of equivalent of 2400 mmol per day to make dogs hypertensive. Moreover sheep given 2% sodium chloride do not develop hypertension (Potter 1972); however, Whitworth et al (1979) did raise the mean arterial pressure in sheep by 13 mmHg but used 340 mmol of saline in the food plus isotonic saline to drink instead of water. All these levels are far above the average daily intake of salt in man in this country. Furthermore, the effect is not

uniform with some animals very hypertensive on high salt diets and some with completely normal blood pressure. Indeed Aoki and co-workers (1972) found no difference in blood pressure of spontaneously hypertensive rats on 2.7% sodium chloride compared to a low salt intake, although the latter may well have rendered the animals malnourished. It is worth noting that the diets fed to many animals precipitated ill-health and often debilitating diarrhoea. Consequently, possibly relevant other factors either were missing from the diet or malabsorbed. Moreover even the enormous salt loads failed to raise pressure immediately. Blood pressure elevation (when present) was an extremely chronic phenomenon: similarly those studies that removed the salt stimulus failed to show an immediate reduction in pressure but an equal prolonged decline. Finally, these extremes of dietary salt excess appear to have little relevance to the range seen within human populations.

High Salt Intake Studies in Man and Primates

One study has reported the effects of a high salt intake in primates (Cherchovich et al 1976). These workers carried out observations on baboons exposed to high salt intake from birth (n=4) (1% wt/vol drinking water and 4% wt/wt food); in animals during the period of sexual maturation (n=16) and as adults (n=9). All animals exposed to a high salt intake showed an increase in mean arterial pressure, but once again the diets were extremely high in sodium. Many of the adult animals placed on the diets suffered ill-health and had to be withdrawn from the study. Nevertheless, this report did demonstrate similar findings in primates that had heretofore only been shown in rats, fowl and rabbits, and recently have been reproduced in monkeys (Srinavasan et al 1984).

Studies in man began over thirty years ago. McDonough and

Wilhelmj (1954) reported the effects of augmenting a normal diet with an average daily supplement of 38 gms of salt in one individual for 23 days. The subject became oedematous and his systolic and diastolic blood pressures rose 40/20 mmHg, and fell when the salt intake was reduced. Once again the amount of salt used was five times a normal daily intake and of course the study took place with only one volunteer. This study was followed by two negative reports using normotensive subjects (Gros et al 1971; Kirkendall et al 1976). Kawasaki et al (1978) chose to divide a group of hypertensive subjects into 'salt sensitive' and 'salt resistant' subjects according to arbitrary criteria and demonstrated reductions in mean blood pressure on a low sodium diet (70 mmol/day) in salt-sensitive subjects. When switched to the high salt intake (240 mmol/day) both groups showed a rise in mean pressure but only back to normal diet levels. The study was not randomized and the statistics are invalid; despite the claims of the authors little happened on the high salt diet.

The experiments of Murray and co-workers (1978) were performed on 8 healthy normotensive volunteers. They were given a constant diet containing 10 mmol of sodium daily for 7 days; this was increased to 300 mmol/day for 3 days and then increased further to 800 mmol/day for 6 days. For the final three days of the study the subjects were hospitalised and received saline infusions to increase intake to 1500 mmol/day. The diet was tolerable provided the volunteers were allowed free access to water, which ameliorated the tendency to develop diarrhoea at the high salt levels. No volunteer developed any adverse effect other than fatigue related to sleeplessness because of nocturia, although some ankle oedema was noted at the very high sodium intake levels. Despite this enormous salt load mean blood pressure rose from 82.6 mmHg on the 10 mmol/day to 83.8 on 300 mmol/day

and only to 89.5 on 800 mmol/day. The infusions raised the pressure to 99.2 mmHg or a rise of 15/20 mmHg for increasing the salt intake 150-fold. More relevant to this discussion was the failure of the pressure to change over the dietary range compatible with Western Man's average daily salt intake. Similar conclusions were reached by Burstyn et al (1980) increasing the sodium intake with a supplement of 250 mmol daily for 8 days. In addition, Sullivan and co-workers (1980) reported an essentially negative study when looking at changing from 10 mmol/day sodium diet to 200 mmol/day. The authors claimed a pressor response but only in borderline salt-sensitive hypertensive subjects.

Proponents of salt being responsible for essential hypertension argue that the human studies are short-term and therefore meaningless when applied to the onset of blood pressure in middle-life, which they contend is the result of a chronic ingestion of excess salt. In some respects this is a position which is difficult to refute. However, compensatory biological systems are more likely to produce adaptation to a pressor stimulus with a chronic fall in blood pressure rather than the reverse. Slow pressor responses are occasionally observed (e.g. to initially sub-pressor doses of angiotensin II) but these are not of the same time scale as that postulated for essential hypertension in man e.g. over years.

In conclusion therefore, I have endeavoured to document the three main sources for linking salt to essential hypertension. I have omitted a discussion on some aspects of the debate (e.g. Hedonistic salt preferences or taste predilections) because significant evidence in favour of a primary abnormality is lacking. On present published data the links between excessive salt intake and essential hypertension are tenuous in the extreme.

Chapter 2

Ion Transport in Relation to Sodium Balance and Hypertension

The investigations of sodium movements in and out of cells in hypertension have produced many conflicting results. Much of the work has been done on circulating blood cells due to the inaccessibility of resistance vessel material for reliable study. Interpretation of these experiments are fraught with difficulties particularly where the tissue studied is the erythrocyte. Before considering the data so far gathered from blood cells in hypertension, I feel that it would be prudent to look at some of the factors that make the understanding of this field particularly difficult.

Erythrocyte Ion Transport

It is now well established, for example, that there are many genetically determined haematological abnormalities that will alter the integrity and characteristics of the erythrocyte membrane per se (Parker and Berkowitz 1983). Hereditary spherocytosis, for instance, is well known to be associated with an elevated intracellular sodium and increased Na^+/K^+ -ATPase activity (Wiley 1972). Homozygotes and heterozygotes for sickle cell disease exhibit reduced red cell $\text{Na}^+ \text{K}^+$ cotransport and raised sodium content (Crook and Mroczkowski 1985). This has obvious implications for studies of negro populations.

It has been known for over 30 years that ethnic background may change intra-erythrocytic sodium content. Love and Burch (1953) noted higher sodium levels in negro blood donors. This finding was later confirmed (Munro-Faure et al 1971) although Nature published this report as if it was novel information. In addition, the abnormalities are not confined to sodium content. Ouabain-sensitive rubidium uptake reflecting Na^+/K^+ ATPase activity is low in normotensive blacks (Woods et al 1981) and such findings have been confirmed by others (Hennessy and Ober

1982). There are also racial differences in sodium influx (Etkin et al 1982) and $\text{Li}^+ \text{Na}^+$ countertransport (described below) (Trevisan 1983(a)). The differences are not merely confined to coloured populations, with discrepant findings also reported in Caucasians from Boston and Paris (Canessa et al 1981), or in ethnically different populations in California (Beutler et al 1983). One study has examined all aspects of erythrocyte sodium handling with respect to race (M'Buyamba-Kubangu et al 1984). Here, sodium was elevated, $\text{Na}^+ \text{Li}^+$ countertransport and rubidium uptake was reduced with sodium influx enhanced in negroes. In addition, the same study reported a reduction in erythrocyte frusemide-sensitive Na^+ efflux (cotransport described below). This has been confirmed by workers in Los Angeles and France (Tuck et al 1984, Garay et al 1981). Tuck also studied hypertensive blacks and whites. In this study sodium pump activity was however not different in normotensive or hypertensive blacks compared to Caucasian controls.

Two further highly relevant aspects are age and sex. In many studies of cation transport in hypertension, the patients are often older as a group. Gambert and Duthie (1983) have compared subjects with a mean age of 31 years with a group aged 76 years. Erythrocyte Na^+/K^+ ATPase activity was significantly reduced in the older group. Brugnara et al (1983) measured erythrocyte $\text{Li}^+ \text{Na}^+$ countertransport in normotensive and hypertensive subjects and demonstrated a negative correlation between age and countertransport in males but not in females. Sex matching also appears to be important: Duhm et al (1982) and Williams et al (1983) noted reductions of both erythrocyte Na^+ cotransport and countertransport systems in women compared to men. A similar

(although not statistically significant) finding has subsequently been reported by Cooper et al (1983).

Because hypertensive patients are often heavier, investigators have examined the effects of weight on cation transport. De Luise et al (1980) reported reduced numbers of erythrocyte sodium pump units, decreased pump activity and increased intracellular sodium. On the other hand, Mir and co-workers (1981) found ATPase activity and ouabain sensitive efflux enhanced.

These considerations apart, erythrocyte sodium transport can be altered in a number of disease states and by a number of hormones (Parker and Berkowitz 1983; Cumberbatch and Morgan 1981). Finally, some studies have used hypertensive patients who have received antihypertensive drugs. Certainly sodium efflux has been shown to fall with diuretic therapy in white blood cells (Araoye et al 1978) and to rise in erythrocytes (Walter 1981).

Any review of the literature on plasma membrane handling of sodium and hypertension, encounters a wide variety of experimental approaches. This extends not only to the species studied but also to the tissues used and even then most reports have focussed on just one facet of sodium transport. In human essential hypertension studies of sodium transport in resistance blood vessels are extremely limited owing to the inaccessibility of this tissue. Much work has been performed on cells easily obtained such as adipocytes or blood cells. It has to be assumed that any differences observed in such cells are representative of what is occurring in vascular smooth muscle. Experiments in animal hypertension allow such comparisons to be made in genetically susceptible animal strains, although whether such abnormalities noted are

relevant to human hypertension is unclear. This section attempts to detail the disturbances reported in sodium transport in hypertension and to examine the hypothesis put forward to explain them.

The links between sodium intake and blood pressure in humans have been examined in the previous chapters. Tissue abnormalities of sodium handling began to emerge shortly after 1950. Tobian and Binion (1952) reported the post-mortem finding of an increase in sodium and water concentrations in renal arteries and psoas muscles of patients who had had essential hypertension. Whilst conceding that the tissues studied were much larger than resistance arterioles, Tobian surmised that if similar findings were to be present in the smaller vessels, luminal diameter would be smaller and hypertension would result as a consequence of "water-logging" leading to increased impedance of blood flow. Tobian and Binion (1954) extended their work to experimental hypertension in the rat, and reported a high sodium content in aortae from DOCA treated hypertensive rats and rats made hypertensive by renal artery ligation compared to control animals. The criticism has been made that the findings at post-mortem were due to artefact occurring as a result of cell death. In addition, the difficulty remains in determining cause and effect i.e. whether changes in vascular tissue are a consequence of hypertension with increased load increasing extracellular binding of sodium. Thus with the problem of obtaining human vascular tissue in life and of identifying secondary changes, workers turned to alternative cell lines. In organising the findings in red blood cells one finds that the field is beset with problems. For example, the internal sodium content is determined by the rate of active clearance of the cation by

the sodium pump. Some workers have measured red cell sodium alone; others have measured this plus one transport pathway such as influx, or transmembrane efflux. Below I have tried to document the findings in hypertension when blood cells have been studied. This field is littered with repetitive reports and so only one publication is cited for each centre.

Internal Sodium Content

Upon this aspect of sodium handling, there appears to be little consensus, evidence is divided on either side with a slight bias in favour of the sodium being normal. Until 1985, there had been 22 reports of the content of sodium in red cells of hypertensive patients. The early reports (D'Amico 1958 and Losse et al 1960) concluded that the sodium content was raised. These were confirmed by (Gessler 1962) and later by Wessels et al in 1967. With increased interest in ionic movements in hypertension, more contemporary reports have repeated this work and obtained similar results (Fadeke Aderounmu and Salako 1979, Urry et al 1980, Poston et al 1981, Clegg et al 1982 and Birks and Langlois 1982). However, these studies must be set against 13 negative reports (Weller 1959, Bracharz et al 1962, Schroeder 1968, Burck 1971, Munro Faure et al 1971, Canessa et al 1980, Walter 1981, Swarts et al 1981, Ibsen et al 1982, Davidson et al 1982, Montanari et al 1984, Wiley et al 1984, Boon et al 1985). It is difficult to see at first glance why the findings should be so discrepant. Of the nine positive studies, it is possible to implicate ethnic influences in the sample population; Aderounmu studies black Africans only and Poston's hypertensive patients were of mixed racial background. Birk's study was performed on patients who had received medication, although

this is not precisely stated. Losse, Gessler and Clegg used untreated hypertensive subjects. The study by Urry employed nuclear magnetic resonance and did not, in fact, report increased erythrocytic sodium, but rather decreased internal binding of sodium in red blood cells. Moreover, similar criticisms could be applied to many of the negative studies - these however remain in the majority. The explanation for these differing reports may well lie in the study of Clegg et al (1982). This report found that the internal sodium concentration was raised in hypertensive patients compared to control subjects. However the values were highest in those subjects with a family history of hypertension. The heterogeneity of subjects with raised blood pressure might suggest that a selection in favour of patients with a family background of raised blood pressure might bias towards a positive result. Family history is certainly of importance when considering this subject as will be seen below.

Erythrocyte Sodium Transport

Following the initial reports of abnormal sodium in blood vessels and blood cells, interest turned to membrane transport of sodium in and out of the cell. Wessels et al (1967) observed that ^{22}Na accumulation from a ^{22}Na -labelled medium was accelerated in erythrocytes from hypertensive patients. This was confirmed by Postnov et al (1977) and subsequently by others (Etkin et al 1982, Poston 1981, Birks and Langlois 1982). In fact, there are no dissenting reports on this subject. In erythrocytes of hypertensive patients the conclusion must be that passive sodium influx is enhanced.

Increased cell membrane permeability to sodium and the consequent rise in the intracellular sodium concentration would normally stimulate sodium efflux through an increased activity of the

energy-dependent ouabain sensitive Na^+/K^+ ATPase sodium pump. However when this has been studied with regard to hypertension further controversy emerges. Some of this can be explained in terms of exactly what has been measured and reported; for example, some studies measure efflux rate constant but do not measure absolute efflux rate, for which a knowledge of internal sodium is essential. Postnov et al (1977) did indeed find that Na^+/K^+ ATPase mediated sodium efflux was enhanced. However, Aderounmu and Salako (1979) found absolute sodium efflux rate lower in red cells from hypertensive patients although this did not attain statistical significance. On the other hand, Fitzgibbon et al (1981) only measured erythrocyte efflux rate constants and found them increased in hypertensives when the cells were incubated in their own plasma. Similarly, Wambach and Helber (1981) found Na^+/K^+ ATPase activity increased in the same tissue and Woods et al (1981) reported enhanced ouabain-sensitive rubidium uptake (which probably reflects sodium pump activity) in hypertension. However Swarts and co-workers (1981) found no abnormalities at all, and Poston (1981) reported a decreased erythrocyte total efflux rate constant and an elevated (though not significant) absolute sodium efflux rate. Similarly, Walter and Distler (1982) found a reduced ouabain sensitive efflux rate constant in hypertension but no change in absolute efflux because intracellular sodium was slightly higher in hypertensive patients although not significantly so. In the red cell therefore the majority of reports indicate enhanced sodium efflux and this appears to be due to stimulation of the sodium pump but not to an increased number of pump sites (Smith et al 1984, Boon et al 1985). Two other carrier-mediated transport pathways

have been investigated in the human erythrocyte. Sodium-lithium countertransport is measured by preloading erythrocytes with lithium and comparing its extrusion into solutions which contain physiological concentrations of sodium or no sodium. The resulting difference probably represents activity of a carrier-mediated system which exchanges intra- and extracellular sodium on a 1:1 ratio. Once again, allowing one publication from each group of workers there are ten reports of this system in hypertension. there is remarkable unanimity in their conclusions: countertransport has been found to be raised in seven reports (Canessa et al 1980; Clegg et al 1980; Cusi et al 1981; Canali et al 1981; Ibsen et al 1982; Trevisan et al 1983(a); Brugnara et al 1983). In one report (Montanari et al 1984) it was raised but did not attain statistical significance and in only two papers (Swarts et al 1981 and Duhm et al (1982) has it been found to be normal. Throughout this literature there is a tendency for the abnormality to be most pronounced in those patients with a family history of hypertension. Moreover, as with all the other defects described in this chapter, no abnormality has been described in secondary forms of hypertension.

The second carrier-mediated transport pathway investigated is the frusemide-sensitive sodium-potassium co-transport system. It is measured as the ratio of sodium extrusion to potassium extrusion in sodium loaded erythrocytes pretreated with ouabain. Six out of eight reports have demonstrated that this system is depressed in essential hypertension (Garay et al 1980; Cusi et al 1981; Ghione et al 1981; Davidson et al 1982; Tuck et al 1983; Montanari et al 1984). The other two studies found no differences (Swarts et al 1981; Wiley et al 1984). Sixteen

out of 52 patients studied by Swarts were receiving therapy and in the study of Wiley 12 patients were on antihypertensive medication and 2 patients were grossly obese.

In summary, therefore, even allowing for poor patient selection, mismatching for age and weight, ethnic background and antihypertensive drugs, one can conclude that in essential hypertension passive influx of sodium into erythrocytes is probably increased. Absolute efflux is increased due to stimulation of the sodium pump and sodium countertransport is raised and cotransport is reduced at least in the majority of populations studied. In the case of the last two systems their net contribution to inward or outward sodium movements is small, if any. The internal cell sodium is normal or slightly raised. Despite claims to the contrary there is considerable overlap with normal values in all these parameters and no test can be viewed as diagnostic of essential hypertension. The fact remains however, that the cell membrane handling of sodium is disturbed although not invariably.

These experiments in essential hypertension have been repeated in the offspring of patients with high blood pressure amongst whom there will be a number at increased risk of developing the disease in later life. An increase in intra-erythrocytic sodium in the normotensive first-degree relatives of hypertensive patients has been noted (Henningsson et al 1979; Gudmundson 1984; Lijnen et al 1984). Henningsson (1979) has also noted increased sodium influx in the same population, although this was not confirmed by Gudmundsson (1984). Total sodium efflux was elevated in the study by Cooper et al (1983b) but decreased in the study of Lijnen et al (1984); Gudmundsson (1984) and Cusi et al (1981) found no change. Woods et al (1982) reported increased rubidium

uptake in erythrocytes of relatives again suggesting an enhanced sodium pump activity. Once again most studies agree with regard to $\text{Na}^+ \text{Li}^+$ countertransport, with an increased rate noted by Canessa et al (1980); Woods et al (1982); Cusi et al (1981) and Ibsen et al (1981). Similarly, co-transport studies have demonstrated decreased activity in these normotensive offspring (Meyer et al 1981, Cusi et al 1981, Lijnen et al 1984). One would expect misclassification of these populations which could therefore be diluted and to find such differences between the groups is perhaps surprising. Nevertheless, the impression is that influx may be raised, internal sodium is raised and efflux is enhanced with raised countertransport and depressed cotransport.

Leucocyte Sodium Transport

In order to be related to any putative cellular mechanisms that might be causing the increased peripheral vascular resistance underlying essential hypertension, the disturbances in cation transport that have been demonstrated, must be representative of those in vascular smooth muscle cells. For this reason some workers have examined sodium movements in leucocytes in hypertension. The tissue used has either comprised lymphocytes or a mixed leucocyte population. The advantage is that the white blood cell is more metabolically active and it is to be hoped will provide a cell line closer to the vascular smooth muscle cell than the erythrocyte. Unlike the red blood cell, for example, the leucocyte is nucleated, can synthesise protein and has 30-40 times the number of sodium pumps located in its plasma membrane. The reports on this tissue in hypertension are limited, and some of the reason for this is undoubtedly in the difficulty

of isolation and utilisation of the cells.

The first report of leucocyte sodium transport in hypertension appeared in 1975 (Edmondson et al). This demonstrated increased intracellular sodium and water and decreased mean efflux rate constant in 17 hypertensive patients. These were matched for sex, race and age (although no details of the control group were given), and only two of the patients had received antihypertensive medication. The results are somewhat difficult to interpret: the reason for this is that the intracellular sodium content was measured at room temperature whereas the rate constant was derived from cells at 37 °C. (In fact, calculating the efflux rates shows that total efflux is again increased in hypertension). However the results for the intracellular sodium are difficult to accept. The standard error of the mean of the hypertensive patients was 8.5 implying a standard deviation of 35 mmol/kg dry weight of cells. The range for the hypertensives must therefore have been 84 to 189 and for the controls 101 to 137. Clearly the error on this measurement renders it extremely insensitive. Nevertheless, total efflux rate constant was reduced and this was due to a decreased ouabain-sensitive activity. No other parameters were measured and as the authors stated no calculation of the absolute transmembrane sodium flux was possible. Araoye and co-workers (1978) confirmed that sodium was raised in a mixed leucocyte population from hypertensive subjects, although the standard deviation results again suggest they are far from perfect. In the same paper, Araoye compared the effects of antihypertensive medication on the cell sodium content and found that diuretics reduced an elevated cell sodium content to normal. He suggested that the elevated sodium was contributing to the

hypertensive state and its reduction had caused pressure to fall. Ambrosioni et al (1979) measured the intralymphocytic sodium in 50 hypertensives, 44 patients with labile hypertension and 40 control subjects. Intralymphocytic sodium was raised in hypertensives and labile hypertensives compared to controls. These results are again difficult to interpret. The ranges for the three groups were hypertensives (26.1-39.8 mmol/kg), labile hypertensives (14.1-40.1 mmol/kg) and controls (15.4-31.4 mmol/kg). As in the study of Edmondson et al (1975) the results imply large differences in cell size or the errors on the method are so high as to make interpretation difficult. Poston et al (1981a) investigated leucocyte sodium transport in 10 patients with essential hypertension. Some had been treated and there were 8 women, and the population was of mixed ethnic background. The results were compared to those from 25 controls for whom no details are furnished. Again the results are astonishing. The intraleucocytic sodium was twice as high in the cells of hypertensives compared to controls. It seems likely that important systematic artefacts contribute to these results. Nevertheless accepting them at face value, once again total efflux rate is enhanced in hypertensives; efflux rate constants are depressed but the absolute flux is increased due to the large amount of sodium in the cells. Again the prime cause is an increased sodium pump activity. These few reports are difficult to interpret; at best they confirm some findings in the erythrocyte where cell sodium was noted to be raised in hypertension, and in addition they suggest that sodium pumping is increased in hypertension. At worst, they are uninterpretable due to errors in measurement or patient/control selections.

Animal Studies

The study of sodium handling by vascular smooth muscle cells has been confined for the most part to experiments using animals such as the rat. These investigations do have the advantage of allowing workers to examine processes before hypertension is established, following the experimental induction of hypertension or after its reversal. For example, using deoxycorticosterone acetate (DOCA) to raise pressure Friedman et al (1975) showed that sodium permeability and efflux were increased in rat tail artery. Similar experiments using aldosterone and rat aorta have confirmed these findings (Overbeck et al 1982) and by sampling tissue above and below an aortic clamp demonstrated increased sodium efflux in aorta on both sides, indicating that enhanced sodium transport was associated with but not caused by hypertension. Friedman's experiments (1975) were concerned with the early phase of mineralocorticoid-induced hypertension, but subsequent investigations have also demonstrated increased sodium transport in the chronic state (Brock et al 1982). Similar findings have now been demonstrated in the spontaneously hypertensive rat (Jones 1973) and in hypertension induced by kidney wrapping or clipping the renal artery (Brock et al 1982; Overbeck and Grissette 1982). The stimulus to this enhanced efflux is probably increased sodium influx; this has been demonstrated in DOCA hypertensive rats and in SH rats, and in some experiments using renal artery clipped hypertensive animals (Jones 1981). Intracellular sodium content is more difficult to assess as much blood vessel sodium lies in the extracellular compartment, or (in large vessels) bound to collagen. Nevertheless intracellular sodium has not been found to be raised in experimentally induced hypertensive animals or in SH rats (Friedman 1983; Aalkjaer et al 1985). Similarly the number

of sodium pump sites does not appear to be different in such animals (Aalkjaer et al 1985) and in rat red blood cells once again the findings are similar. In erythrocytes from animals treated with DOCA, sodium influx, internal sodium and efflux were enhanced (Duhm et al 1983), and these parameters were also increased in renal artery clipping experiments (Duhm et al 1983). In SH rats efflux is also increased (Ben Ishay et al 1975). There are two reports of experiments using thymocytes (a leucocyte equivalent) in rats. In one, (Jones et al 1981) intracellular sodium was elevated in spontaneously hypertensive rats and the claim was made that the sodium correlated with blood pressure, however, no account was made for the effects of ageing and when this is considered (Bradlaugh et al 1984) the association disappears. In SH rats absolute efflux again appears to be enhanced (Bradlaugh et al 1984).

These studies were carried out in synthetic media whilst therefore intrinsic changes in vascular ion transport can be detected, it is still theoretically possible that plasma factors create differences in vascular function between hypertensive animals and their controls. Thus Haddy and Overbeck (1976) postulated that volume expanded forms of experimental hypertension are associated with suppressed sodium pump activity due to a circulating inhibitor in the blood. Negative results might be explained by such factors being washed off. However little evidence from animals or humans substantiates this viewpoint.

In conclusion, there appears to be remarkable concordance in the work to date on cell membrane handling of sodium in hypertension both in animal blood vessels and in blood cells from animals and humans. The influx and efflux rates for sodium are enhanced. The significance of this phenomenon is discussed in the next section.

Sodium and Vascular Reactivity

As I have emphasised above, the link between dietary sodium and essential hypertension has arisen from a knowledge that in some forms of renal impairment excessive sodium intake can lead to raised blood pressure. Moreover, the preceding data provide evidence for cell membrane handling of sodium being abnormal in hypertension. The main question left to consider is whether sodium can effect changes in vascular smooth muscle contraction and hence influence peripheral resistance and blood pressure?

Insight into this particular question had to wait until 30 years ago. Leonard (1957) found that removal of external K^+ or treatment with cardiac glycosides caused an increase in tension in arterial strips. Mason and Braunwald (1964) extended these findings and demonstrated that peripheral vascular resistance in normal man could be increased by low doses of digitalis, although the changes were admittedly short-lived in both experiments. Either removing external K^+ or the use of cardiac glycosides reduces the activity of plasma membrane Na^+/K^+ ATPase pumps and leads to an increase in internal sodium content (Glynn 1964). The contractures observed come about as a result of a decrease in calcium efflux (Reuter et al 1973) and an increase in calcium influx (Van Breemen et al 1973) which occur in response to the withdrawal of sodium. Therefore, the inter-relationship between sodium and calcium appears to be well established; indeed in vascular smooth muscle, calcium extrusion appears to be sodium-dependent at least in part. In cardiac muscle cells the relationship is even closer with the demonstration of a $Na^+:Ca^{2+}$ exchange mechanism now known to have a significant role not only in determining the contractility of heart muscle but under some conditions

possibly affecting membrane potential and the shape of the cardiac action potential (Brading and Lategan 1985). However, the existence and importance of a physiological $\text{Na}^+ - \text{Ca}^{2+}$ exchange in smooth muscle remains controversial (Brading and Lategan 1985, Aaronson and Van Breemen 1981). What is not in doubt is that in healthy humans and animals infusion of cardiac glycosides results in an acute increase in peripheral resistance (Dock and Tainter 1930, Ross et al 1960). It has been established that part of this is due to increased noradrenaline release, but even in the presence of ganglionic blockade resistance increases suggesting that direct effects on vascular smooth muscle cells also occur (Ross et al 1960). As stated above the effect is often short-lived and may even be followed by vasodilatation in some vascular beds (Vatner et al 1971, Mulvany et al 1982). In view of the possibility that the vasodilatation might be centrally mediated, workers have infused ouabain into the forearm (Robinson et al 1983). These experiments showed a vasoconstrictor effect lasting one hour or more, not influenced by α -adrenoceptor blockade. Moreover, infusing K^+ into the forearm decreased vascular resistance (Robinson et al 1983) indicating that stimulating of the Na^+/K^+ ATPase pump can acutely reduce tone in blood vessels. This is supported by the finding that inhibition of the pump by ouabain abolishes this effect (Anderson et al 1983).

As indicated above, inhibition of Na^+/K^+ pump activity probably causes vasoconstriction by two mechanisms, one neurogenic and the other a direct effect on the smooth muscle cells. The neurogenic effect is due to noradrenaline release from nerve terminals stimulated by a rise in sodium in nerve endings, reducing calcium extrusion via the $\text{Na}^+:\text{Ca}^{2+}$ exchange mechanisms. This

would then cause free calcium to rise (Nakazato et al 1980) and stimulate catecholamine release. The myogenic response to sodium pump inhibition is brought about either by a similar interaction between sodium and calcium or as a result of the depolarization which occurs at the time of pump inhibition.

The question remains as to how these theories may be applied to essential hypertension? If the sodium pump were instrumental itself in elevating peripheral vascular resistance it would have to be underactive: in pumping 3Na^+ out for each 2K^+ pumped in the resulting outward current must contribute to the membrane potential, but inhibition would be pre-requisite for depolarization. Thus, for excess sodium intake to be responsible for hypertension it would require an unphysiological response by the sodium pump in smooth muscle cells. Two theories have been postulated. The first implicates a humoral factor in essential hypertension, and was formally proposed by Blaustein (1977) and developed by de Wardener and MacGregor (1980). These workers extended the concepts of Dahl (1969) who proposed that a circulating saluretic substance might cause a sustained rise in arterial pressure in salt-sensitive rats. Following the experiments of Dahl, Haddy and Overbeck (1976) developed this hypothesis to include blood pressure elevations that occurred with volume expansion, including primary aldosteronism, cortisone administration, chronic renal failure and some forms of experimental hypertension. De Wardener and MacGregor postulated that subjects with essential hypertension possessed a genetic defect of the kidney which impaired salt excretion. This was aggravated throughout life by a diet too high in salt; the resulting salt excess leads to sodium and water retention and a consequent expansion of

the blood volume. In response to this volume increase, the hypothalamus secretes a hormone with ouabain-like properties which promotes sodium excretion of the sodium pump in kidney tubules. They also propose that this factor also inhibits the sodium pump in vascular smooth muscle. The result of this would be an increase in intracellular sodium and according to Blaustein (1977) subsequent interference with sodium calcium transmembrane exchange. The resulting rise in intracellular calcium would increase smooth muscle tone and raise pressure.

This hypothesis is undoubtedly supported in part by some of the reports of reduced ouabain sensitive efflux rate constant in blood cells of hypertensive patients. However, it fails to explain many of the ouabain-independent mechanisms that are disturbed in patients, such as influx, co-transport and countertransport mechanisms. Moreover it can be questioned on a variety of other points, quite apart from the "salt" factor which has already been detailed above.

Whilst it has been known for some years that increased renal perfusion results in a natriuresis and diuresis in the presence of normal renal function (Selkurt 1951), unless there is a compensatory change in renal sodium conservation as a result of high blood pressure, patients would rapidly dehydrate. Thus to maintain sodium balance in hypertension the relationship between sodium excretion and perfusion pressure must be shifted to the right (Guyton and Coleman 1969; Brown et al 1974). If this shift were to be the primary event, then the early phase of essential hypertension would indeed be associated with sodium retention and volume expansion as de Wardener and MacGregor claim, these abnormalities being corrected when a new steady

state was achieved at the expense of an increased systemic blood pressure. If the relationship were initially normal then hypertension should result in sodium and water loss with reductions in plasma and extracellular fluid volumes. Certainly as de Wardener and MacGregor acknowledge "In man with established essential hypertension evidence that there is an expanded extracellular fluid space is as difficult to detect as it is in the hypertensive rat, once hypertension is established." The consistent finding in essential hypertension is that plasma volume is reduced (Tarazi et al 1968; Safar et al 1976; Bing and Smith 1981). The situation in early essential hypertension is more difficult to define with certainty because of the risk that some subjects studied ultimately remain normotensive (Lund-Johansen 1983). Nevertheless there is no evidence for blood volume expansion. It is possible that the explanation for this is that changes occur in the partitioning of fluid between the different body compartments which is not reflected in overall changes in fluid volume. Indeed, in a later modification of their hypothesis de Wardener and MacGregor (1982) argue in favour of cardiopulmonary volume diversion as a primary event. In borderline hypertension Safar et al (1974) have claimed that there is a redistribution of fluid from the systemic to the pulmonary circulation. Even accepting the possibility occurring, from a physiological standpoint, the phenomenon must be considered of dubious importance in the pathogenesis of hypertension. If it is the result of a selective increase in sympathetic efferent activity, it may be a manifestation of the primary abnormality and not necessarily a mediator of hypertension. In this regard a similar cardiopulmonary diversion occurs in sodium depletion (Ferrario et al 1981) where autonomic activity

is increased. Moreover if diversion is a result of increased pulmonary vein complacance, pulmonary venous pressure will not rise and so it is difficult to see how cardiac output and blood pressure can be altered (Birkenhager and de Leeuw 1984). However, the evidence is against expansion of blood volume due to excess sodium and water in the early stages of hypertension. If anything a slight reduction in exchangeable sodium and extracellular fluid has been reported in mildly hypertensive and young hypertensive subjects (Bing and Smith 1981, Bauer and Brooks 1982, Beretta-Piccoli et al 1982). Thus the stimulus for the secretion of a ouabain-like factor appears to be reduced rather than increased in the early stages of the disease.

If the putative humoral factors does exist, its glycoside-like action must be able to increase peripheral vascular resistance. Short term studies described above would suggest that such contraction as could be elicited by ouabain may be short-lived, although the effects of repetitive stimulation may, of course, theoretically raise blood pressure by initiating structural hypertrophy in blood vessels. Interference with a $\text{Na}^+:\text{Ca}^{2+}$ exchange mechanism and its significance in vascular smooth muscle remains speculative (Brading and Lategan 1985).

The alternative hypothesis regarding disturbances of membrane sodium handling suggests that they are manifestations of a genetic abnormality of the physicochemical structure and function of the plasma membrane of the cell, influencing independently a variety of electrolyte transport mechanisms and altering blood pressure by such mechanisms such as changing intracellular calcium not directly reliant upon any univalent ion transport abnormalities (Swales 1982, Heagerty et al 1985).

My studies outlined below are designed to test the hypothesis of de Wardener and MacGregor by examining sodium movements in hypertensive patients and their normotensive offspring, and by observing blood pressure and sodium transport whilst manipulating sodium balance by changes in dietary salt intake or pharmacological means.

Chapter 3

Methods

The experiments described were performed on leucocytes obtained from human subjects by peripheral venesection. The leucocyte was studied in preference to the erythrocyte because the former cell is nucleated, respire aerobically and is able to synthesise protein, properties not possessed by red blood cells. In addition, leucocytes are highly metabolically active and possess far more Na^+/K^+ ATPase pump sites than erythrocytes. If such work is to have any bearing upon possible abnormalities underlying the disease process causing essential hypertension, the cell line investigated must have characteristics similar to vascular smooth muscle cells. In this regard, the leucocyte appears a better cell to study than the erythrocyte.

Leucocyte Ion Transport

The assays developed in the laboratories in the Department of Medicine at Leicester allowed the measurements of intraleucocytic sodium content and sodium efflux. The methods employed were based on modified protocols adopted from reports by Baron and Ahmed (1969) and Hilton and Patrick (1973). Baron and Ahmed (1969) outlined the isolation of white blood cells from whole blood, measurement of the trapped extracellular water and estimations of intracellular electrolyte content. Hilton and Patrick (1973) developed these methods to allow not only a knowledge of intracellular sodium to be obtained but also transmembrane movements of sodium to be calculated using radioisotopes. A list of equipment and solutions used is given in Appendix I.

Isolation of Leucocytes

Venous blood was collected from an antecubital vessel using the vacutainer system (Becton Dickinson, Rutherford, New Jersey). This system allows the collection of blood via a 19 gauge needle

into tubes containing lithium heparin as an anticoagulant with minimum frothing. Total efflux and intracellular sodium studies are performed using 80 mls of blood, but in some experiments described below, the volume used differed slightly. No details are furnished by Baron on the equipment he used to collect samples, but in view of the sometimes large quantities collected, the vacutainer system appeared to be the most convenient.

Sedimentation

In the original experiments of Baron and the subsequent experiments of Hilton, dextran solution was added in the proportion of one part to four parts of blood (v/v), and mixed by gentle inversion five times with care to avoid frothing. However, dextran cannot be used at 37 °C; I felt that if my experiments were to have any relevance to the situation in-vitro, all the procedures should be executed at 37 °C. For this reason I sought to find a solution that could be used at body temperature. It was therefore decided to use plasmagel (Uniscience, Cambridge, UK). This was added in 7.5 ml aliquots to sterile containers (Sterilin, Herts, England). To this was added 15 ml of anticoagulated whole blood. Between 4 and 6 sterilins would be used for each experiment. Plasmagel had a further advantage of containing sodium in a concentration of 150 mmol/l, unlike dextran. Extracellular sodium did not alter dramatically from that seen in the venous blood from whence the cells had been taken. The sterile containers stood upright in a waterbath at 37 °C for 25 minutes, at which time the supernatant plasma (approximately 15 ml) containing leucocytes, some erythrocytes and platelets were removed with a plastic Pasteur pipette, leaving undisturbed the sedimented erythrocytes. The plasma was transferred to plastic Sarstedt

centrifuge tubes and stoppered.

Lysis of Remaining Erythrocytes

The stoppered tube was centrifuged at 37 °C at a rate of 1000 rpm (300 g) for 7.5 minutes. After removal from the centrifuge the supernatant was discarded and the cell pellet left at the base of the conical centrifuge tube. This appears red due to a mixture of erythrocytes and leucocytes. Two ml of distilled water was added to the tube and exactly 13 seconds later 2 ml of X2 Earle's solution was added to restore isotonicity. Timing is crucial; in a series of experiments that I originally performed I found that delaying the addition of the buffer by more than 5 seconds led to death of the leucocytes. With correct mixing and timing all the erythrocytes could be haemolysed without damaging the leucocytes (Baron 1963). In his original experiments Baron used an unbuffered x10 concentration tissue culture medium diluted to x4. The pH of this solution is 3.2, and I felt it was inappropriate to use this acid fluid. In place of this, I used X2 Earle's buffer gassed with 95% O₂/5% CO₂ mixture just before use to give a pH of 7.4 (Appendix I).

Final Leucocyte Isolate

The suspension of cells was then centrifuged at 300 g for 5 minutes at 37 °C. The supernatant was again discarded and the cell pellet left at the base of the tube. This appeared as a pure yellowish button of leucocytes resembling pus, often with a thick rim of red on the top which comprised erythrocyte ghosts. The number of leucocytes thus obtained was calculated by using a Coulter Counter, and found to be 50.2% per ml of that observed in unprocessed venous blood (n=4). The differential white cell count was estimated in duplicate in four healthy

volunteers and found to be similar to that observed in peripheral venous blood (Table 1). The percentage of smear cells was similar to that seen when examining peripheral blood films. Morphologically the leucocytes appeared normal in shape and size. Tests of viability were performed using a dye impermeability test (Wilson and Manery 1949). This employed a drop of 0.5% aqueous solution of Trypan blue, added to leucocytes in tissue culture medium 199 and incubated for 30 minutes. Viability was indicated by failure to take up dye. In six experiments, viability was always greater than 95%.

Percentage of Water in Pellet

In a series of seven experiments (Table 2) the percentage of water in the cell pellet was estimated. This was achieved by weighing a small aluminium foil sack before beginning the experiment, again when containing the pellet wet, and again after drying the sack overnight in an oven at 100 °C. The percentage of water in the pellet was calculated by the formula:

$$\text{Percentage} = \frac{\text{wet wt of pellet} - \text{dry wt of pellet}}{\text{wet wt of pellet}} \times 100$$

The duplicate mean percentages of the seven experiments (Table 2) were similar to those obtained by Baron and Ahmed (1969).

This water is divided between that contained within the cells and that trapped outside. In order to estimate the percentage of fluid trapped between the cells an extracellular label was employed. Thus while preparing the leucocyte cell pellet as above, 0.02 ml of radioiodinated human serum albumin was added to the final cell suspension, and the cells isolated as before. Following the final centrifugation a few drops of the supernatant

fluid were placed in a sealable small conical plastic 1 ml tube (Sarstedt Germany); the supernatant fluid sample and the final cell pellet were sealed to avoid water loss by evaporation. The tubes were preweighed and reweighed to ascertain the weights of pellet and supernatant. The radioactivity of the tubes was counted in an auto-gamma spectrometer (Packard, New York, USA). The activity of the extracellular fluid was calculated and the exact proportion of extracellular fluid in the cell mass was derived. From the figures the true wet weight of the leucocyte mass can be obtained and the percentage of water in the extracellular compartment estimated. The water content of the final total leucocyte isolate (H_2O_t) was estimated by oven drying (as shown above). The quantity of extracellular fluid (wt_e) was derived by ^{131}I -HSA method. The ECT contains dissolved solids and the actual water content is 97% (Baron and Ahmed 1969). The calculated water content of ECF (H_2O_e) = $Wt_e \times 97$. From these values the true water content in leucocyte intracellular fluid was calculated ($H_2O_i = H_2O_t - H_2O_e$). The difference between the total weight of the leucocyte isolate (Wt_t) and the weight of ECF (Wt_e) gives the true wet weight of the leucocytes (Wt_i).

$$\text{Percentage content of water in leucocytes} = \frac{H_2O_i}{Wt_i} \times 100$$

In a series of six experiments the mean percentage of water in leucocytes was $66 \pm 0.92\%$ which was not different from the value obtained by Baron and Ahmed (1969).

Intraleucocytic Electrolytes

The original method for estimating intracellular leucocyte sodium and potassium content was initially employed (Baron and Ahmed 1969). This entailed the isolation of leucocytes from

30 ml of venous blood as outlined above, and transferring the cells to a preweighed polythene tube using lay-flat tubing (Hedley and Co., Leytonstone, England). After centrifuging the cell suspension at 300 g for 3 minutes the supernatant was removed and the inside of the tube dried with paper tissues and the cell pellet ashed in an oven for 12 hours at 100 °C. The tube was then reweighed and the sodium and potassium estimated by flame photometry. The figures obtained in this way were inaccurate and reproducibility poor. After much experimentation I came to the conclusion that the principal source of error was the problem of weighing when using a lay-flat polythene tube of 400 mg to hold a cell pellet weighing approximately 5 mg when oven-dried. It was necessary therefore to modify the method.

In consequence, the leucocyte pellet was obtained as outlined above and suspended in 3 ml M199 in a conical Sarstedt tube and placed in a waterbath at 37 °C for 20 minutes. At the end of this time the tube was centrifuged at 37 °C at 300 g for 3 minutes. The supernatant was removed and the inside of the tube dried with tissue paper and the cells resuspended in 3 ml of ice-cold magnesium chloride (99 mmol). This solution was employed to wash off excess sodium from the outside of leucocytes and paralyse the sodium pump. The tube and contents were centrifuged for 3 minutes at 300 g at 4 °C and the supernatant removed and the pellet dispersed in 1 ml of magnesium chloride. The suspension was then transferred to a sterile plastic 1 ml Sarstedt pipette tip, which had been heat-sealed at its tip. This had been previously shown to contain no potassium or sodium. The tip and contents were centrifuged at 900 g for 3 minutes at 4 °C. The supernatant was removed and the inside of the tip carefully dried as before.

The end of the tip was then carefully cut off using a scalpel blade and the cell pellet transferred by gentle blowing using a syringe into a preweighed sack. This sack was made from aluminium kitchen foil carefully cleaned with alcohol and contained no sodium. The average weight of the sack was 9 mg and thus drastically reduced the difference between the weight of the dried pellet and its container. The sack was dried in an oven at 100 °C for 12 hours and reweighed to give the dried weight of the cells. Following reweighing, the sack and contents were placed in a 2 ml Sarstedt tube and 1.5 ml of deionised water added to them to leech out the electrolytes from the ash. After 24 hours aliquots in duplicate were taken from the tube and the sodium measured using a flame photometer (Corning, UK) and lithium standards. Table 3 illustrates the intracellular sodium and potassium contents of duplicate aliquots from nine separate experiments. The coefficient of variation was 5% and 3.5% for sodium and potassium respectively.

Leucocyte Sodium Efflux Rate Constant

The method employed to measure the efflux rate constant for sodium was modified from that described by Hilton and Patrick (1973). Following isolation of leucocytes from 60 ml venous blood as before the cell pellet was resuspended in 6 ml of M199 in a conical 12 ml Sarstedt plastic tube. To this was added 5 uCi of ^{22}Na and the tube placed in a waterbath for 25 minutes at 37°C to equilibrate. At the end of this time, the tube was placed in a centrifuge at 37 °C and spun for 3 minutes at 300 g.

The supernatant was removed and the cells washed with 6 ml of M199 without radioactivity added and replaced in the centrifuge and spun at 37 °C for 3 minutes at 300 g. The pellet was then

resuspended in a further 6 ml of M199 and divided into two, with 3 ml being placed into a further Sarstedt conical tube. To one of these was added 0.1 ml of ouabain (1 mmol/L) and at 0, 10 and 20 minutes 1 ml aliquots of both cell suspensions were taken and placed in 1 ml centrifuge tubes. These were centrifuged at 900 g for 3 minutes and the supernatant removed with a pipette and the inside of the tubes dried with a tissue. The cell pellets were counted for residual radioactivity using an auto-gamma counter (Packard). The sodium efflux rate constant for the leucocyte was calculated from the regression line of the natural logarithm of radioactivity unit dry weight⁻¹ on time, which was a linear function.

I found, however, that there was considerable error on this method as described above. Hilton measured the weight of each cell pellet after oven drying them. Because of my problems in accurate weighing of such small pellets I corrected my results by standardising them for the protein content in each cell plug by using the method of Lowry et al (1951). To carry this out, the cell plugs were dissolved in 1500 μ l of 1N sodium hydroxide. Following this, an aliquot of the sample was taken and the protein content measured spectrophotometrically using a standard curve. Total efflux rate constant was calculated from linear regression analysis on the aliquots taken without ouabain having been added. Ouabain resistant efflux rate constant was calculated from the linear regression analysis performed on ouabain-treated aliquots. Subtracting these values gave a measure of ouabain sensitive efflux rate constant. The r value for all experiments was in excess of 0.9; if an experiment failed to provide such a value it was rejected. Table 4 illustrates the results of the efflux

rate constants for five experiments performed in duplicate. There was no significant difference in any parameter when a student's paired 't' test was applied. The coefficients of variation were: total ERC 8.5%; ouabain resistant ERC 16%; ouabain sensitive ERC 13.3%.

Absolute Leucocyte Sodium Efflux Rate

Absolute sodium efflux rates were calculated from a knowledge of intraleucocytic sodium content and efflux rate constant. The product of these two values provides the efflux rate (mmol per kg dry weight hr^{-1}). This parameter gives an index of sodium flux out of the cell either via all active and passive mechanisms in the case of total efflux, via ouabain resistant pathways or by the sodium pump (ouabain sensitive efflux). Table 5 shows the results of four pairs of experiments. The coefficients of variation on the assay were: total efflux 6%; ouabain resistant efflux rate 20%; ouabain sensitive efflux rate 11%.

Day to Day Assay Variation

In 12 subjects the interassay variations were measured on leucocyte sodium movements recorded on two occasions separated by a minimum of 7 days (Table 6). The relative coefficients of variation were:

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

Ouabain sensitive efflux rate: 24%

The ouabain resistant efflux rate constant had a high coefficient of variation which was again reflected in the absolute ouabain resistant sodium flux. The main purpose of the experiments was to study ion transport by the sodium pump however for which the methods appeared of acceptable reproducibility.

Measurement of Plasma Renin Activity (PRA)

Ten ml of blood is taken into cooled vacutainers containing 150 mg of dipotassium ethylene diamine tetra-acetate. PRA is measured by radioimmunoassay of generated angiotensin I according to the method of Sealey et al (1974) except that phenyl methyl sulphonyl fluoride is used as an enzyme inhibitor during incubation.

Table 1' Mean + SEM Differential White Cell Count (%) of Final Leucocyte Isolate

	(n=4)		
	Aliquot 1	Aliquot 2	Mean of Aliquots
Neutrophils	70 $\pm$ 3	70 $\pm$ 3	70 $\pm$ 3
Lymphocytes	18 $\pm$ 3.7	17 $\pm$ 3.2	17.5 $\pm$ 3.4
Eosinophils	5 $\pm$ 1	5 $\pm$ 1	5 $\pm$ 1
Smear cells	9 $\pm$ 1.4	9 $\pm$ 1.5	9 $\pm$ 1.4

Remaining cells: Basophils and monocytes only observed in 2 subjects.

Table 2 Percentage of Water in Pellet

Experiment	Sample A	Sample B
1	79.4	77.7
2	82.0	80.3
3	78.8	79.1
4	81.5	79.5
5	80.2	79.7
6	81.1	80
7	79.4	78.8
Mean $\pm$ SEM (%)	80.3 $\pm$ 0.5	79.3 $\pm$ 0.33

Table 3 Mean Intracellular Sodium and Potassium Contents (mmol/kg dry weight of cells) for Duplicate

Leucocyte Samples from 9 Experiments

Experiment	Sample 1		Sample 2	
	(Na)	(K)	(Na)	(K)
1	69	310	62	327
2	47	337	45	340
3	76	316	72	348
4	39	337	38	317
5	48	343	46	323
6	31	298	32	311
7	36	341	31	344
8	38	288	36	286
9	30	319	32	320
Mean $\pm$ SEM	46 $\pm$ 5.4	321 $\pm$ 6.6	44 $\pm$ 4.8	324 $\pm$ 6.4

Table 4 Values of Leucocyte Efflux Rate Constants (h^{-1}) in 5 Duplicate Experiments

Experiment	Sample 1			Sample 2		
	Total ERC	OR ERC	OS ERC	Total ERC	OR ERC	OS ERC
1	1.776	0.572	1.204	1.902	0.356	1.546
2	1.716	0.349	1.367	1.722	0.404	1.318
3	2.418	0.774	1.644	2.85	0.882	1.968
4	1.878	0.502	1.376	1.644	0.478	1.166
5	2.124	0.536	1.588	1.944	0.678	1.266
Mean $\pm$ SEM	1.98 $\pm$ 0.13	0.55 $\pm$ 0.07	1.44 $\pm$ 0.08	2.01 $\pm$ 0.22	0.56 $\pm$ 0.1	1.45 $\pm$ 0.14

(ERC=Efflux Rate Constant, OR=Ouabain Resistant, OS=Ouabain Sensitive).

Table 5 Absolute Leucocyte Sodium Efflux Rate (mmol/kg/h^{-1}) in 4 Pairs of Experiments

Total	Ouabain Resistant	Ouabain Sensitive	Total	Ouabain Resistant	Ouabain Sensitive
87	28	59	88	27	61
71	19	52	59	17	42
53	11	42	55	13	42
85	27	55	87	16	71
Mean $\pm$ SEM					
74 $\pm$ 7.9	21 $\pm$ 4.0	53 $\pm$ 3.9	72 $\pm$ 8.8	18 $\pm$ 6.1	54 $\pm$ 7.2

Table 6(a) Leucocyte Sodium Movements in 12 Subjects on 2 Different Days

Day 1											
Subject	Total ERC (h ⁻¹)	OR ERC (h ⁻¹)	OS ERC (h ⁻¹)	(Na) (mmol/kg dry wt cells)	(K) (mmol/kg dry wt cells)	Total Flux (mmol/kg dry wt cells h ⁻¹)	OR Flux (mmol/kg dry wt cells h ⁻¹)	OS Flux (mmol/kg dry wt cells h ⁻¹)			
1	2.142	0.702	1.440	42	394	90	29	61			
2	1.662	0.570	1.092	35	353	58	20	38			
3	1.902	0.378	1.524	55	336	105	21	84			
4	3.204	0.798	2.406	50	354	160	40	120			
5	1.962	0.906	1.056	40	346	78	36	42			
6	1.752	0.258	1.494	49	384	86	13	73			
7	1.770	0.240	1.530	30	380	53	7	46			
8	1.806	0.522	1.284	37	388	67	19	48			
9	1.974	0.738	1.236	45	367	89	33	56			
10	2.058	0.756	1.302	37	326	76	28	48			
11	1.878	0.738	1.140	57	344	107	42	65			
12	1.476	0.438	1.038	58	369	86	25	31			
Mean±SEM	2.0±0.12	0.6±0.06	1.4±0.1	45±2.7	362±6.3	88±8.1	26±3.1	59±7			

Table 6(b) Leucocyte Sodium Movements in 12 Subjects on 2 Different Days

Day 2

Subject	Total ERC (h ⁻¹)	OR ERC (h ⁻¹)	OS ERC (h ⁻¹)	(Na) (mmol/kg dry wt cells)	(K) (mmol/kg dry wt)	Total Flux (mmol/kg dry wt cells h ⁻¹)	OR Flux (mmol/kg dry wt cells h ⁻¹)	OS Flux
1	2.070	0.408	1.662	41	362	85	17	68
2	2.124	0.678	1.446	35	360	74	24	50
3	1.776	0.708	1.068	39	324	69	28	41
4	3.378	0.702	2.676	40	330	135	28	107
5	2.310	0.636	1.674	40	352	92	25	67
6	1.992	0.456	1.536	42	336	84	19	65
7	1.884	0.300	1.584	33	355	62	10	52
8	1.758	0.546	1.212	36	336	63	20	43
9	1.470	0.320	1.152	30	327	44	10	34
10	2.040	0.540	1.500	40	342	82	22	60
11	2.082	0.670	1.410	49	357	102	33	69
12	1.968	0.702	1.266	52	438	102	37	65
Mean±SEM	2.1±0.13	0.56±0.04	1.5±0.12	40±1.8	352±8.7	83±6.8	23±2.4	60±5.5

Chapter 4

Leucocyte Sodium Transport Studies in Essential
Hypertensive Patients, Normotensive Offspring of
Essential Hypertensive Patients and Matched Controls.

The experiments in this Chapter were performed on normal control subjects, subjects with untreated essential hypertension and normotensive first-degree relatives of essential hypertensive patients. In all subjects leucocyte sodium efflux rate constants and intracellular electrolytes were measured so that data on absolute efflux rates for sodium could be calculated. Blood pressure was always measured using a Hawksley Random Zero Sphygmomanometer by myself. Three readings were obtained in the supine position and three in the standing position, and the mean of the readings in each position recorded. On the day of study, subjects were also weighed and measured for height. Blood was drawn into lithium heparin tubes and assays performed as outlined above. All experiments were commenced between 0900 and 1000 hours to minimise the chance of diurnal hormone variations influencing results. I performed all the experiments myself.

Normotensive Control Subjects

A total of 49 individuals were studied. All subjects had no family history of blood pressure and the group's physical characteristics are shown in Table 1. As can be seen the males were significantly older, taller and heavier than the females, and as a result both supine and standing blood pressures were significantly higher in the males. Table 2 shows the mean leucocyte sodium transport characteristics of the group as a whole and divided into males and females. Intracellular sodium tended to be higher in females but this did not attain statistical significance. Figure 1 illustrates the sodium efflux rate constants for the whole group, and Figure 2 shows the individual intraleucocytic sodium contents. Both these figures demonstrate that the data are not normally distributed being skewed upwards suggesting

a log normal distribution. For this reason it is inappropriate to use anything other than non-parametric statistics and I have therefore analysed results using the Mann-Whitney U test.

Examining the group as a whole, there was a significant negative correlation between intracellular sodium content and age ($r=-0.357$, $p<0.05$, Fig 3) and between intraleucocytic potassium content and age ($r=-0.454$, $p<0.01$). There was no correlation between any other parameter of leucocyte sodium transport and blood pressure, height, weight or age. There was an increased intraleucocyte sodium in females (Table 2) which increased absolute efflux rates for sodium but none of these measurements attained statistical significance. The reason for the increased sodium was considered to be the younger ages of the females and when the females were compared to a group of age-matched males the difference in intracellular sodium content was lost (Table 3). There appeared to be no differences in leucocyte sodium transport attributable to sex alone.

These data suggest that in any studies of leucocyte sodium transport in essential hypertension it is important to have an appropriate control group well matched for age.

Essential Hypertensive Patients

Twenty-six patients were studied, none of whom had received any medication for raised blood pressure. Hypertension was defined as blood pressure in excess of 160/95 mmHg on three separate measurements. All subjects had been thoroughly investigated to exclude secondary causes of hypertension. These patients were compared to 26 control subjects matched for age, height and weight (Table 4).

Results

There were no significant differences between the physical characteristics of the hypertensive and control subjects (Table 4). Hypertensive patients were slightly older and heavier but neither parameter attained statistical significance. Leucocyte sodium transport characteristics did not differ between the two groups: the mean efflux rate constants for sodium were slightly lower in hypertensive patients (Table 5, Fig 4) but did not attain statistical significance. Intracellular sodium content was slightly higher in cells from hypertensive patients but this was not significant (Table 5, Fig 5). Absolute efflux rates for sodium were not higher in hypertensive patients and not significantly different from controls (Table 5). In the control group, intraleucocytic sodium again negatively correlated with age ($r=-0.416$, $p<0.05$) as did intraleucocytic potassium ($r=-0.59$, $p<0.01$). There were no such correlations in the hypertensive patients. In control subjects, intraleucocytic potassium negatively correlated with supine diastolic pressure ($r=-0.41$, $p<0.05$), standing systolic pressure ($r=-0.6$, $p<0.01$) and standing diastolic pressure ($r=-0.415$, $p<0.05$). Intraleucocytic sodium negatively correlated with supine systolic pressure ($r=-0.53$, $p<0.01$). There were no correlations between intracellular electrolytes and blood pressure in hypertensive patients but there was a tendency for the patients with a family history of the disease to show lower rate constants and high sodium content than those with no such pedigree. (Figs 4 and 5)

Normotensive Relatives of Hypertensive Patients

Offspring of hypertensive patients were defined as subjects who had one or more first-degree relatives known to be on therapy for essential hypertension. Where there was doubt the blood

pressure or history of the relatives was checked by myself or by a family practitioner. If doubt remained the subjects were not studied.

Twenty-five relatives were studied and compared with 25 control subjects matched for age, weight, sex and height (Table 6). There were no significant differences between the two groups with regard to the physical characteristics (Table 6). Total leucocyte sodium efflux rate constant was significantly depressed in the relatives ($p < 0.05$, Fig 6, Table 7). This was attributable to reductions both in ouabain resistant and sensitive sodium efflux rate constants (Fig 6, Table 7) although neither component was significantly reduced. Intraleucocytic sodium was slightly higher in relatives than controls and total efflux rates for sodium were slightly lower but none attained statistical significance (Table 7). The pattern observed in relatives was similar to that seen in essential hypertensive patients and no correlations between intracellular sodium content or rate of sodium efflux and blood pressure were observed in either population. The degree of reduction in sodium efflux rate constant was similar in both groups. In the young control group recruited to match the relatives, there was a significant correlation between age and total efflux rate constant ($r = -0.646$, $p < 0.001$) and between age and ouabain resistant efflux rate constant ($r = -0.634$, $p < 0.001$). These correlations were not observed within the group of relatives.

Table 1 Physical Characteristics of Control Subjects

	Males		Females		All	
N	28		21		49	
Age (yrs)	40 $\pm$ 4.2 (p=0.03)		29 $\pm$ 2.1		35 $\pm$ 2.6	
Wt (kg)	74 $\pm$ 2.3 (p=0.001)		59 $\pm$ 1.7		68 $\pm$ 1.8	
Ht (m)	1.8 $\pm$ 1.1 (p=0.001)		1.6 $\pm$ 1.2		1.7 $\pm$ 0.14	
BP (mmHg)	Lying	Standing	Lying	Standing	Lying	Standing
	137 $\pm$ 3.6**	126 $\pm$ 3.3*	124 $\pm$ 2.5	117 $\pm$ 2.4	131 $\pm$ 2.5	122 $\pm$ 2.2
	72 $\pm$ 2.5	80 $\pm$ 1.6	70 $\pm$ 2.4	78 $\pm$ 1.9	71 $\pm$ 1.7	79 $\pm$ 1.2

** p<0.01

* p<0.05

Table 2 Leucocyte Sodium Efflux Rate Constants, Intracellular Sodium and Potassium Contents and Absolute Efflux Rates for Sodium in Control Subjects

	Males	Females	All
	(28)	(21)	(49)
Total ERC	2.05±0.1 (NS)	2.1±0.12	2.1±0.1
Ouabain resistant ERC (h^{-1})	0.7 ±0.1 (NS)	0.7±0.2	0.7±0.05
Ouabain sensitive ERC (h^{-1})	1.41±0.1 (NS)	1.4±0.12	1.4±0.08
(Na) (mmol/kg dry wt cells)	42 ±2.2 (NS)	49 ±3.6	45 ±2.0
(K) (mmol/kg dry wt cells)	353 ±7.7 (NS)	351 ±8.3	352 ±5.6
Total efflux rate (mmol/kg dry wt cells h^{-1})	88 ±7.4 (NS)	102 ±8.3	94 ±5.6
Ouabain resistant efflux rate (mmol/kg dry wt cells h^{-1})	29 ±3.4 (NS)	34 ±3.6	31 ±2.5
Ouabain sensitive efflux rate (mmol/kg dry wt cells h^{-1})	59 ±6.3 (NS)	68 ±6.6	63 ±4.6

Table 3 Aged-Matched Comparison of Leucocyte Sodium Transport in Males and Female Control Subjects

	Males	Females
N	21	21
Age (yrs)	30 $\pm$ 2.7	29 $\pm$ 2.1
Total ERC (h^{-1})	2.04 $\pm$ 0.14	2.1 $\pm$ 0.12
Ouabain resistant ERC (h^{-1})	0.6 $\pm$ 0.07	0.7 $\pm$ 0.2
Ouabain sensitive ERC (h^{-1})	1.44 $\pm$ 0.1	1.4 $\pm$ 0.12
(Na) (mmol/kg dry wt cells)	45 $\pm$ 2.5	49 $\pm$ 3.6
Correlation between (Na) and age	r=-0.41 (NS)	r=0.08 (NS)

Table 4 Physical Characteristics of Hypertensive Patients and Matched Control Subjects

	Hypertensive Patients	Control Subjects
N	26	26
Males	21	19
Females	5	7
Age (yrs)	51±2.1 (NS)	45±39
Height (m)	1.72±2.0 (NS)	1.74±1.7
Weight (kg)	74±2.8 (NS)	70±2.8
BP (mmHg)		
	Supine	Lying
	182±4.4***	136±3.5
	174±3.9***	126±3.5
	107±2.0***	73±2.2
	110±1.83***	80±2.0

*** p<0.001

Table 5 Leucocyte Sodium Efflux Rate Constants, Intracellular Sodium and Potassium Contents and Absolute

Efflux Rates for Sodium in Control Subjects and Essential Hypertensive Patients

	Control Subjects	Hypertensive Patients
N	26	26
Males	19	21
Females	7	5
Total Efflux Rate Constant (h^{-1})	2.24 $\pm$ 0.14	2.00 $\pm$ 0.08 (p=0.38 NS)
Ouabain Resistant ERC (h^{-1})	0.75 $\pm$ 0.07	0.75 $\pm$ 0.05 (p=0.96 NS)
Ouabain Sensitive ERC (h^{-1})	1.51 $\pm$ 0.13	1.26 $\pm$ 0.07 (p=0.2 NS)
(Na ⁺) (mmol/kg dry wt cells)	42 $\pm$ 2.9	44 $\pm$ 1.9 (p=0.2 NS)
(K ⁺) (mmol/kg dry wt cells)	345 $\pm$ 8.0	350 $\pm$ 6 (p=0.73 NS)
Total sodium efflux rate (mmol/kg dry wt cells h^{-1})	96 $\pm$ 9.5	90 $\pm$ 5.8 (p=0.85 NS)
Ouabain resistant sodium efflux rate (mmol/kg dry wt cells h^{-1})	31 $\pm$ 3.9	34 $\pm$ 2.8 (p=0.31 NS)
Ouabain sensitive sodium efflux rate (mmol/kg dry wt cells h^{-1})	66 $\pm$ 7.6	56 $\pm$ 4.6 (p=0.62 NS)

Table 6 Physical Characteristics of Normotensive Relatives of Essential Hypertensive Patients and Matched

Controls

	Control Subjects	Relatives of Hypertensive Patients
N	25	25
Males	11	11
Females	14	14
Age (yrs)	25+1.1	24+1.02
Wt(kg)	66+2.8	66+2.4
Ht (m)	1.7+2.0	1.69+2.1
BP (mmHg)	Lying	Supine
	Standing	Standing
	129+3.2	126+2.3
	69+2.5	76+1.9

Table 7 Leucocyte Sodium Efflux Rate Constants, Intracellular Sodium and Potassium Contents and Absolute

Efflux Rates for Sodium in Relatives of Essential Hypertensive Patients and Matched Controls

	Control Subjects	Relatives of Hypertensive Patients
N	25	25
Males	11	11
Females	14	14
Total sodium efflux rate constant (h^{-1})	2.26 $\pm$ 0.1	p<0.05 1.96 $\pm$ 0.08
Ouabain resistant sodium efflux rate constant (h^{-1})	0.73 $\pm$ 0.05	NS 0.64 $\pm$ 0.05
Ouabain sensitive sodium efflux rate constant (h^{-1})	1.5 $\pm$ 0.1	NS 1.3 $\pm$ 0.08
Intraleucocytic sodium (mmol/kg dry wt cells)	46 $\pm$ 2.1	NS 49 $\pm$ 3.2
Intraleucocytic potassium (mmol/kg dry wt cells)	357 $\pm$ 7.8	NS 362 $\pm$ 4.4
Total sodium efflux rate (mmol/kg dry wt cells (h^{-1}))	103 $\pm$ 8.4	NS 97 $\pm$ 7.7
Ouabain resistant sodium efflux rate (mmol/kg dry wt cells h^{-1})	33 $\pm$ 2.9	NS 31 $\pm$ 2.9
Ouabain sensitive sodium efflux rate (mmol/kg dry wt cells h^{-1})	70 $\pm$ 6.8	NS 66 $\pm$ 6.7

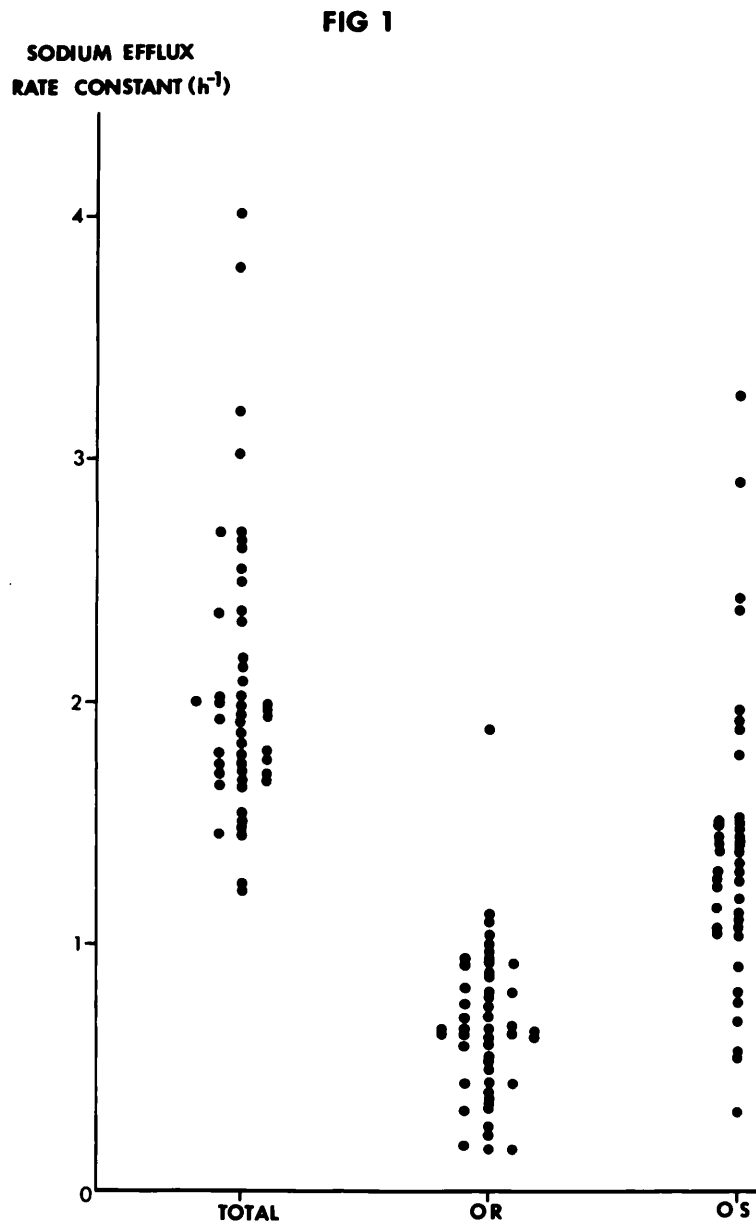


Figure 1: Total, ouabain resistant (OR) and ouabain sensitive (OS) sodium efflux rate constants (ERC) (h^{-1}) in normal control subjects.

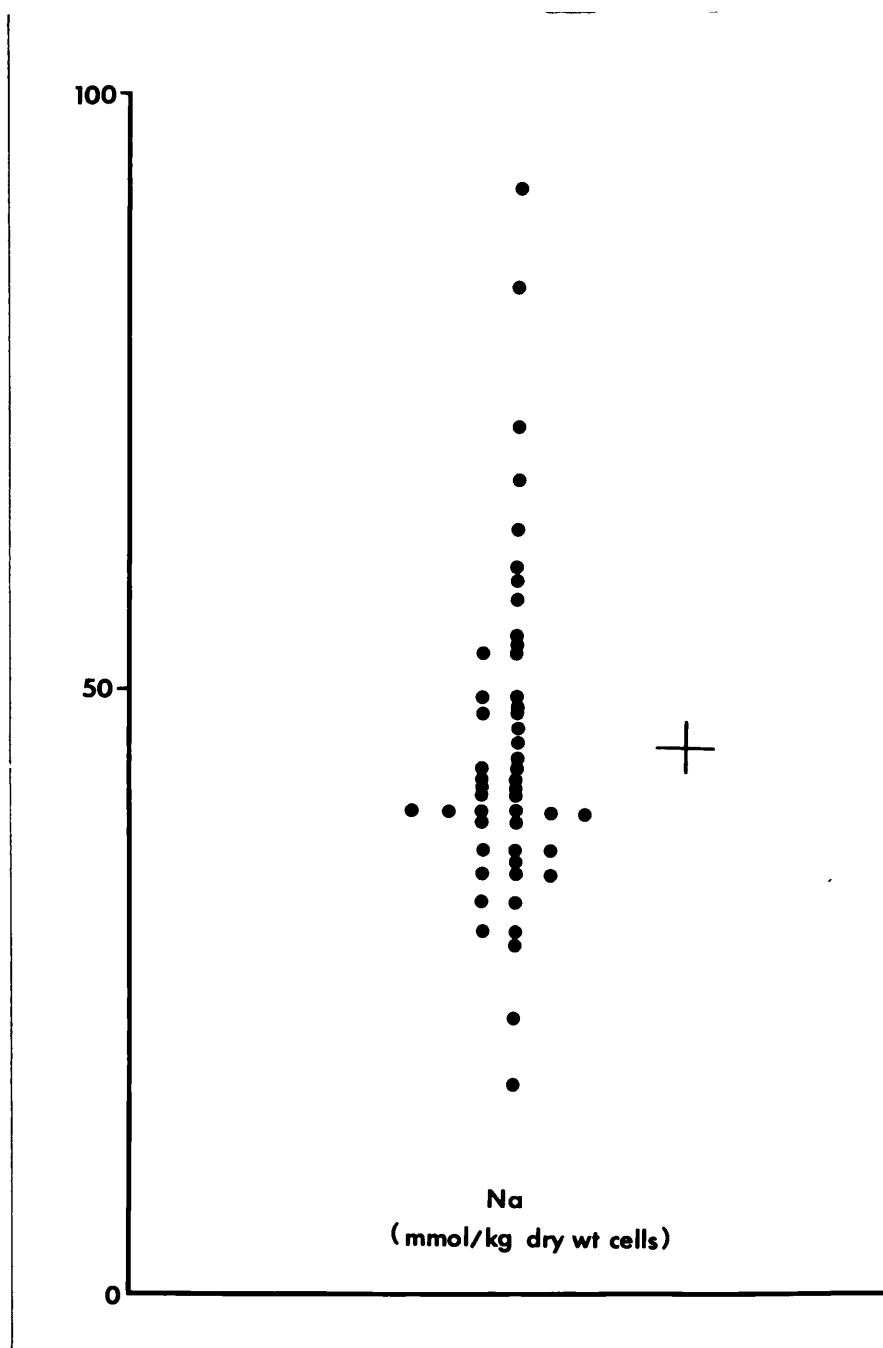


Figure 2: Intraleucocytic sodium content (mmol/kg dry wt cells) in control subjects.

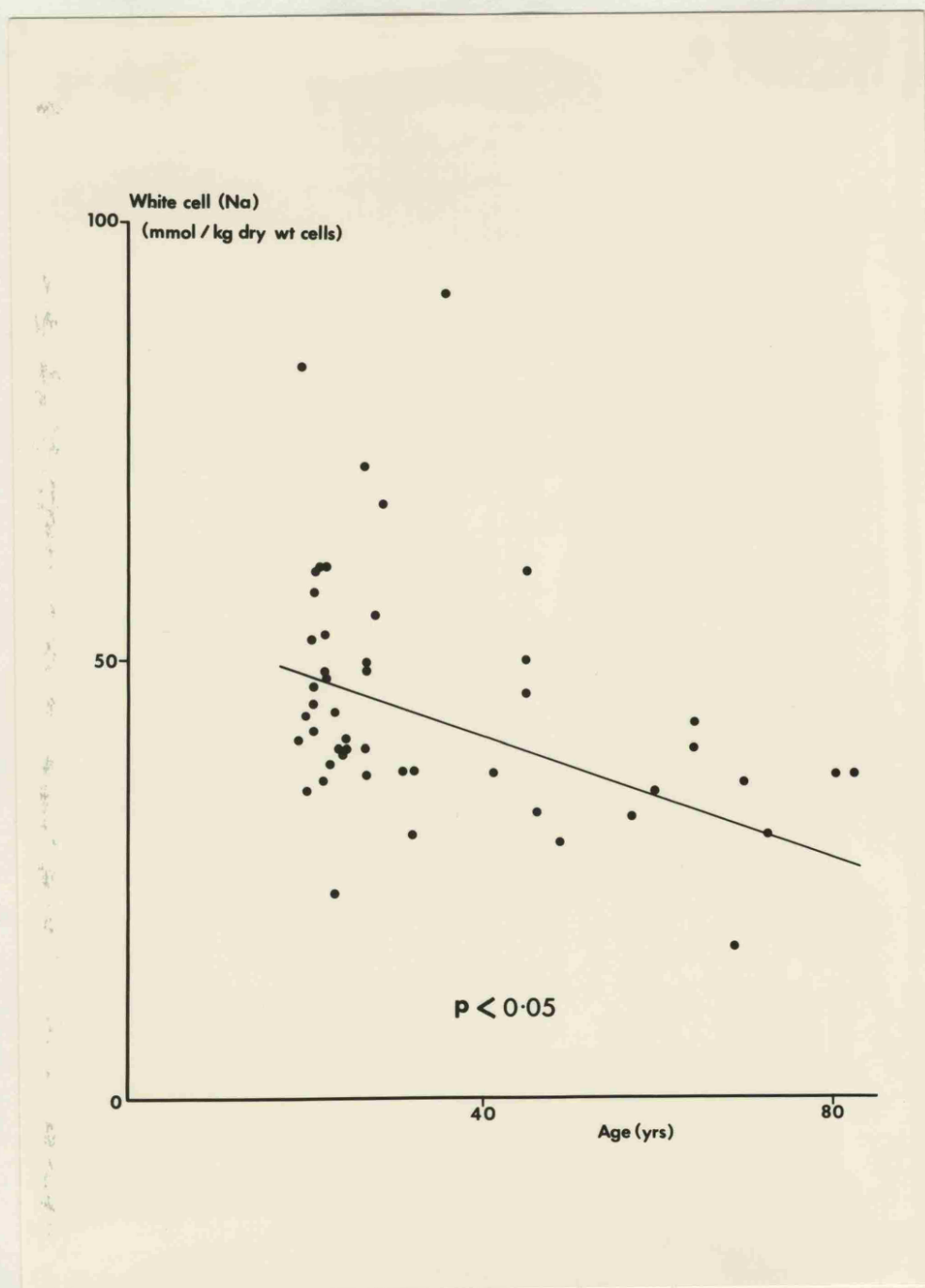


Figure 3: Correlation of intracellular sodium content and age in control subjects.
($r = -0.357$)

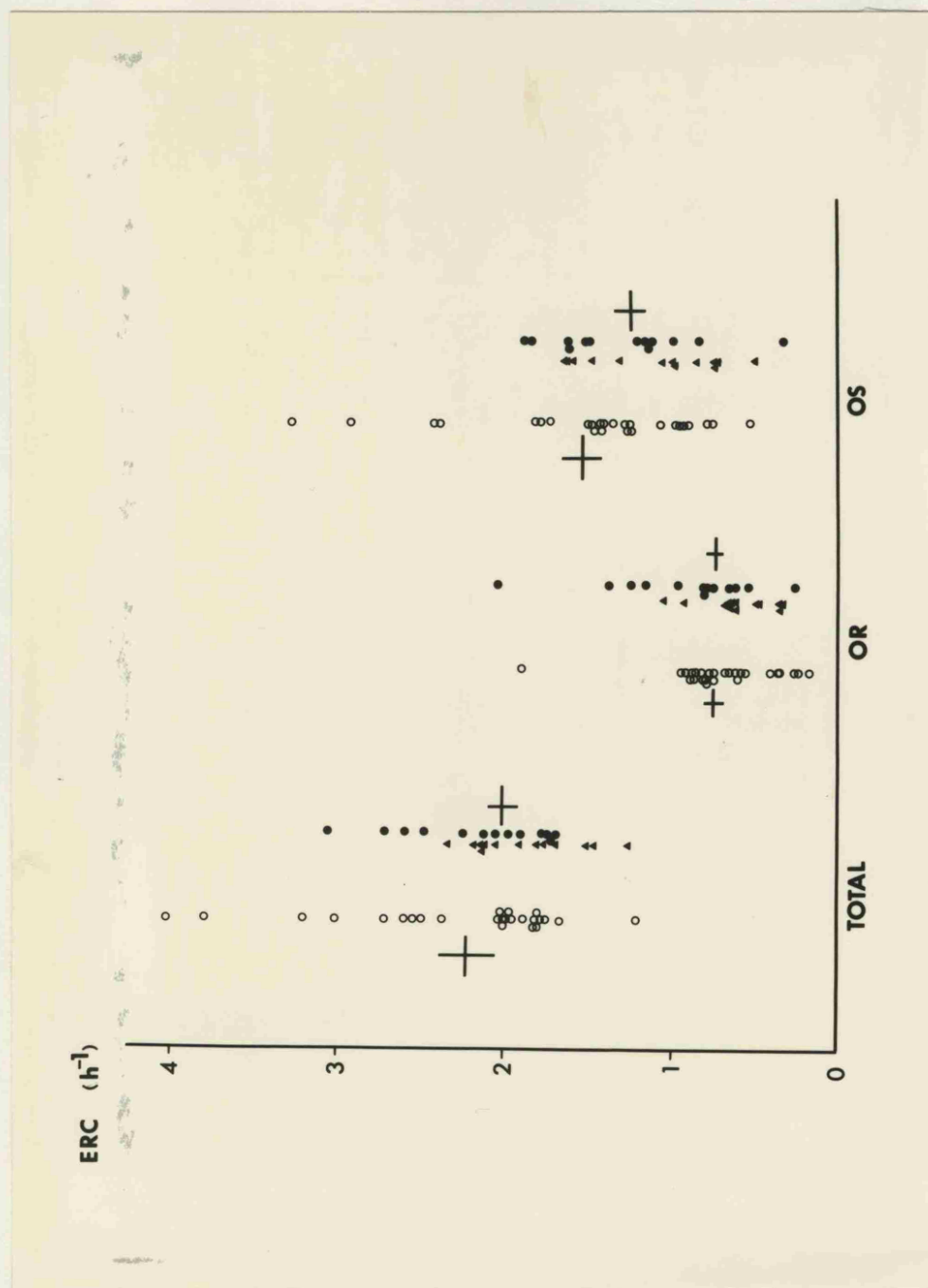


Figure 4: Total, ouabain resistant (OR) and ouabain sensitive (OS) sodium efflux rate constants (ERC) (h^{-1}) in hypertensive patients ((●) without family history (●) with family history) and matched control subjects (○).

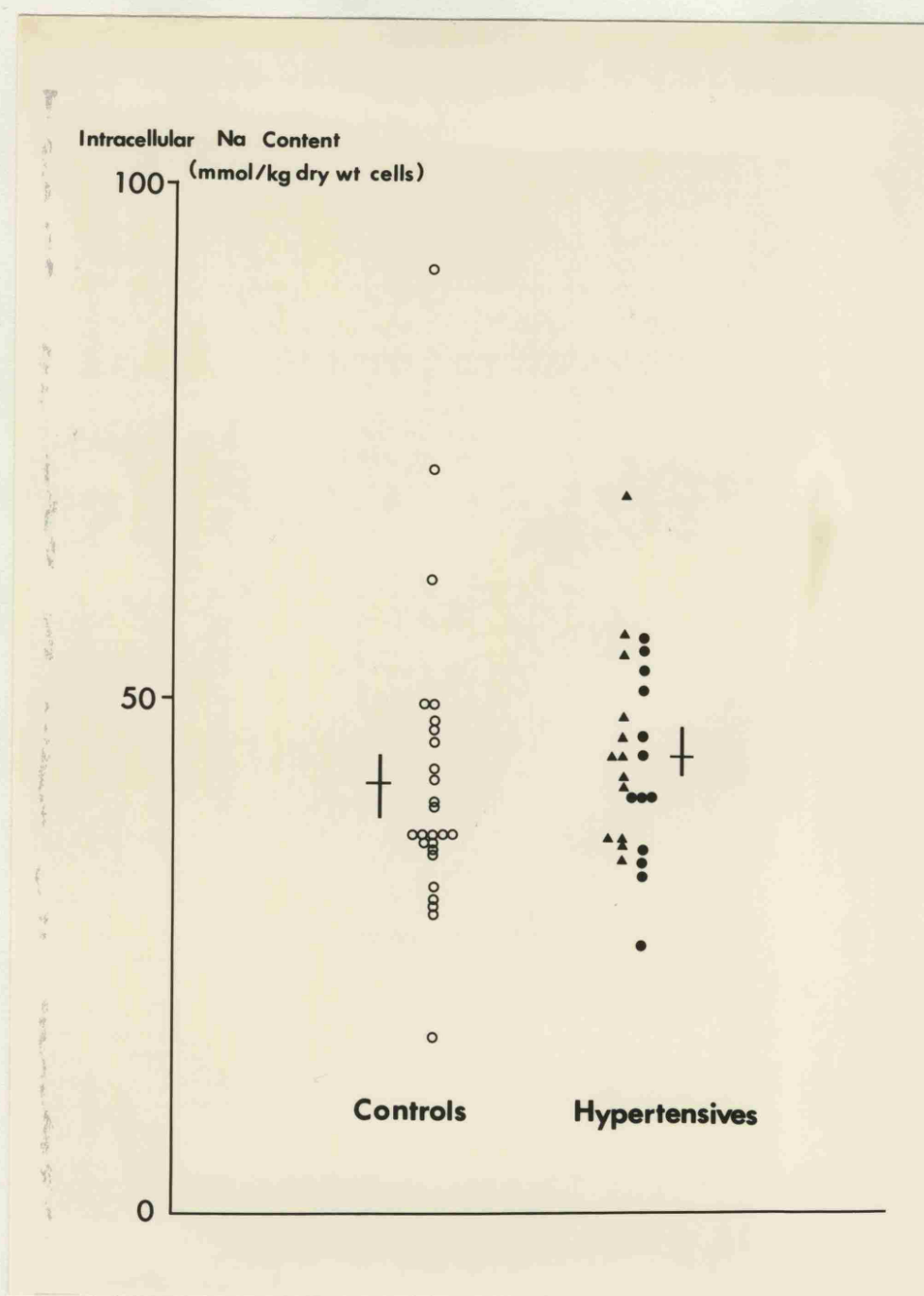


Figure 5: Intraleucocytic sodium content (mmol/kg dry wt cells) in hypertensive patients ((●) without family history (▲) with family history) and matched control subjects (○).

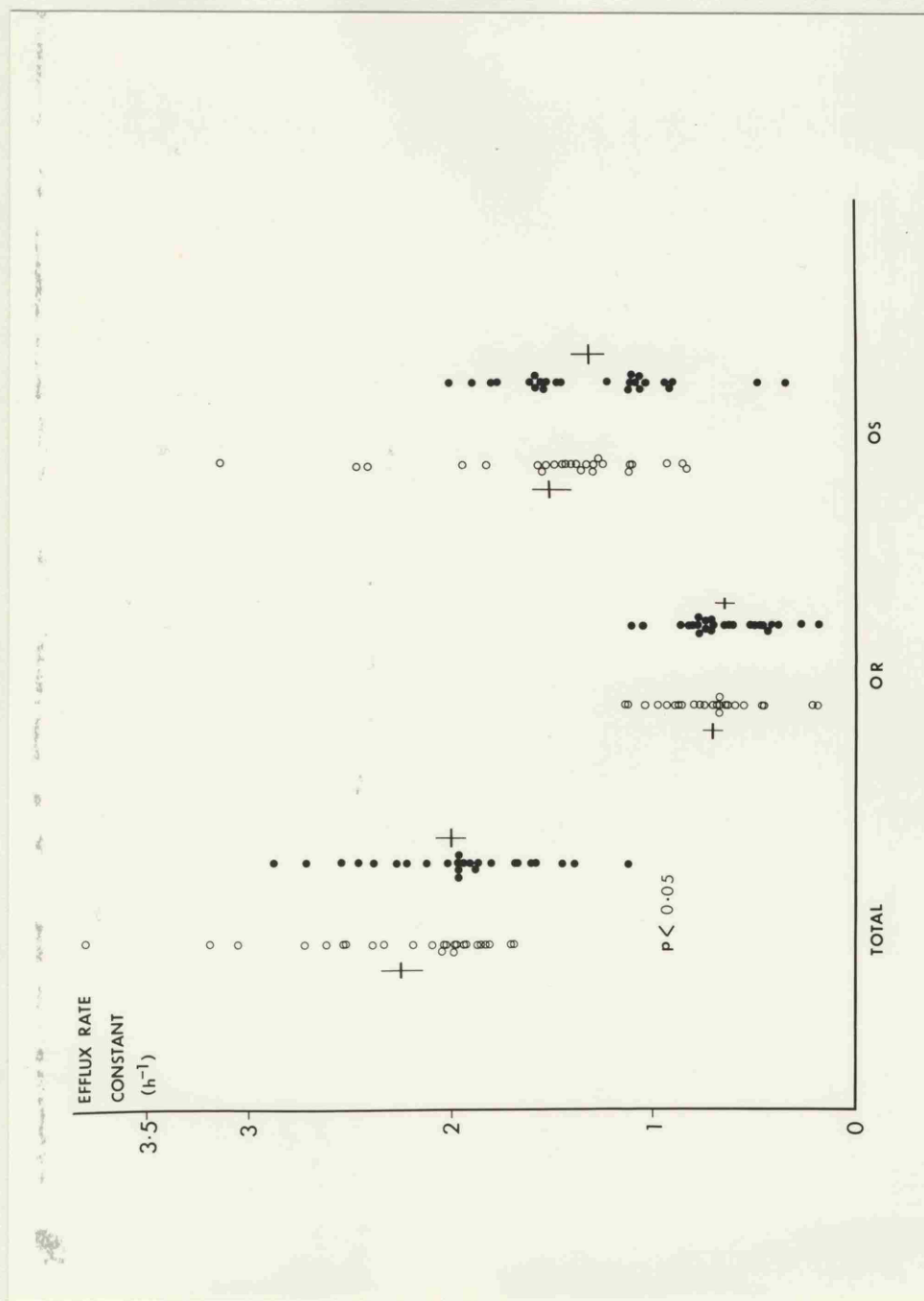


Figure 6: Total, ouabain resistant (OR) and ouabain sensitive (OS) sodium efflux rate constants (ERC) (h⁻¹) in normotensive (●) relatives of hypertensive patients and matched control subjects (○).

Introduction

To have any relevance at all, the abnormalities outlined in the preceding chapter must be representative of similar changes in vascular smooth muscle cells. Indeed, the use of blood cells to study membrane transport processes reflects the difficulty in obtaining human vascular tissue and then producing meaningful results given the complexity of the various constituents of blood vessel walls. Recently it has been possible to study cellular sodium metabolism in resistance vessels (Aalkjær et al 1985). It was therefore decided to investigate whether membrane sodium movements could be correlated in leucocytes and resistance vessels from the same subjects.

Subjects

Chapter 5

Eighteen patients scheduled for elective surgical procedures requiring laparotomy had the following characteristics: age 31 $\pm$ 2.9 years and mean blood pressure 140/82 $\pm$ 2/80 $\pm$ 2.7.

Cell Membrane Sodium Transport: A Correlation

Between Human Resistance Vessels and Leucocytes.

Leucocytes were obtained by peripheral venous puncture just before induction of anaesthesia or during surgery, and resistance vessels were obtained from a biopsy of omentum taken at the time of operation. All subjects gave informed consent and the study was approved by the local ethical committee.

Methods

The measurement of leucocyte efflux rate constants was performed as described above (Methods Chapter). Measurements of sodium transport in resistance vessels were performed using the method of Aalkjær et al (1985). Omental biopsies were placed in a physiological salt solution (PSS) and brought to the laboratory where 5 to 11, 1 mm segments of artery with a diameter of approximately 200 μ m were dissected out for flux determinations. For the flux measurements,

Introduction

To have any relevance at all, the abnormalities outlined in the preceding Chapter must be representative of similar changes in vascular smooth muscle cells. Indeed, the use of blood cells to study membrane transport processes reflects the difficulty in obtaining human vascular tissue and then producing meaningful results given the complexities of the various constituents of blood vessel walls. Recently it has been possible to study cellular sodium metabolism in resistance vessels (Aalkjaer et al 1985). It was therefore decided to investigate whether membrane sodium movements could be correlated in leucocytes and resistance vessels from the same subjects.

Subjects

Eighteen patients were studied of whom 15 were male; the mean age was 63 ± 2.9 years and mean blood pressure was $149 \pm 5.2/80 \pm 2.7$ mmHg supine and $140 \pm 5.5/82 \pm 3.5$ mmHg standing. All subjects had been admitted for elective surgical procedures necessitating laparotomy. Leucocytes were obtained by peripheral venesection just before induction of anaesthesia or during surgery, and resistance vessels were obtained from a biopsy of omentum taken at the time of operation. All subjects gave informed consent and the study was approved by the local ethical committee.

Methods

The measurement of leucocyte efflux rate constants was performed as described above (Methods Chapter). Measurements of sodium transport in resistance vessels were performed using the method of Aalkjaer et al (1985). Omental biopsies were placed in a physiological salt solution (PSS) and brought to the laboratory where 8 to 12, 3 mm segments of artery with a diameter of approximately 200 μ m were dissected out for flux determinations. For the flux measurements

the vessels were kept in PSS at 37 °C for one hour and then loaded in PSS with ^{22}Na (1 $\mu\text{Ci/mol}$) at 37 °C for 30 min. After the loading the vessels were washed for 13 min by taking them through a series of vials each containing PSS at 37 °C. The last vial (11 min to 13 min) contained 1 mM ouabain (Sigma). The rate constants for the total efflux, ouabain resistant and ouabain sensitive flux were then calculated from the washout at specific times. The geometric means of the 8-12 determinations of the total, the ouabain resistant and the ouabain sensitive rate constants were then taken as representative for an individual.

Results are expressed as mean $\pm$ SEM and correlations were obtained by plotting values for leucocytes and blood vessels from the same subject against each other.

Results

Efflux Rate Constant

The mean sodium efflux rate constants (ERC) for resistance vessels in 18 patients were: total $6.93 \pm 0.6 \text{ h}^{-1}$; ouabain resistant $3.3 \pm 0.3 \text{ h}^{-1}$; ouabain sensitive $3.5 \pm 0.4 \text{ h}^{-1}$.

The mean sodium ERC for leucocytes in the same 18 patients were: total $2.06 \pm 0.17 \text{ h}^{-1}$; ouabain resistant $0.66 \pm 0.09 \text{ h}^{-1}$; ouabain sensitive $1.4 \pm 0.15 \text{ h}^{-1}$.

There was a significant correlation between total sodium ERC in the resistance vessels and leucocytes ($r=0.48$, $p < 0.05$, Fig 1). There was no correlation between ouabain resistant ERC ($r=-0.18$, $p > 0.1$, Fig 2), but there was a highly significant correlation between ouabain sensitive ERC in the two tissues ($r=0.64$, $p < 0.01$, Fig 3).

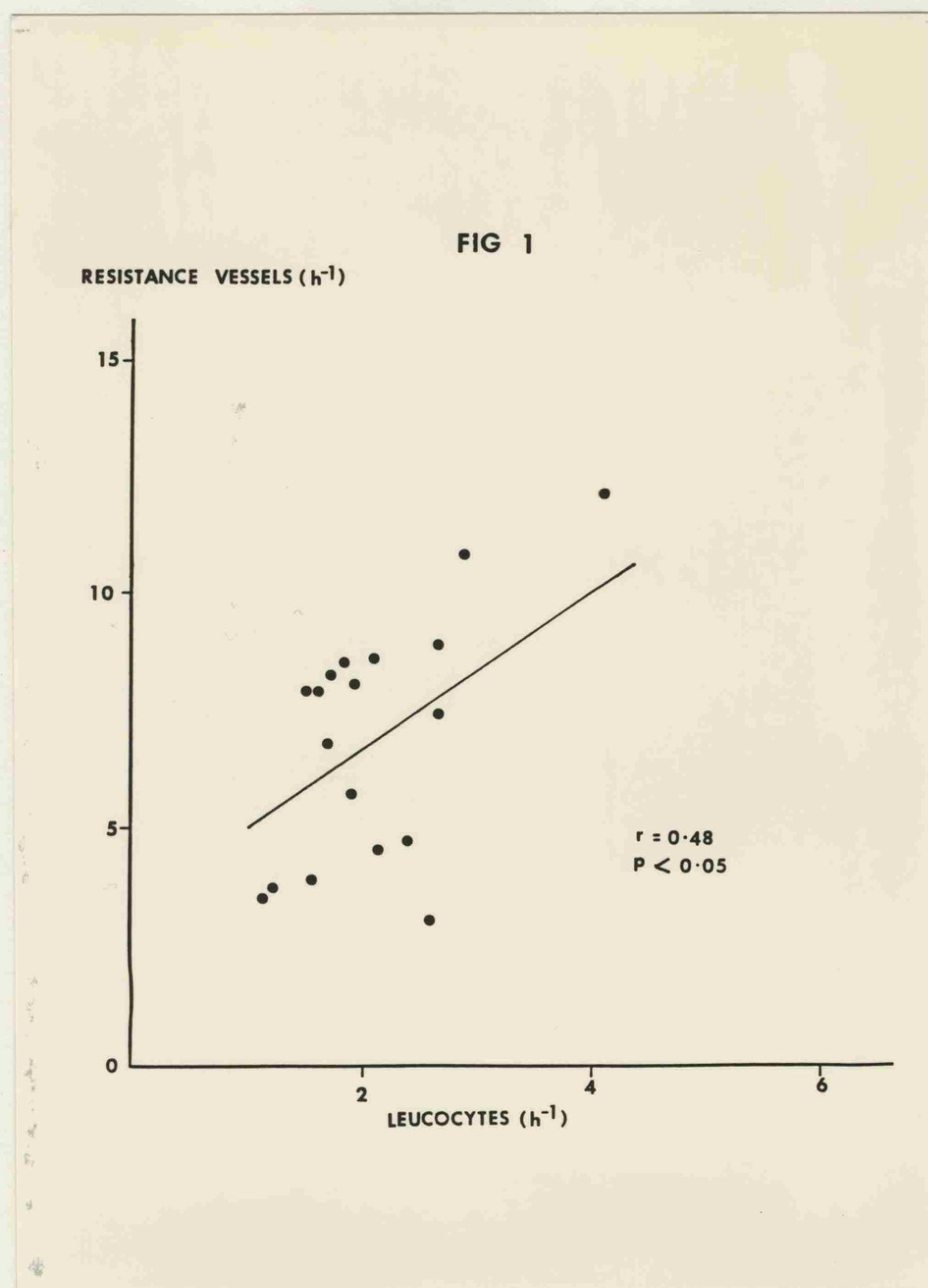


Figure 1: Correlation of total ERC in resistance vessels and leucocytes.
(ERC: Efflux rate constant).

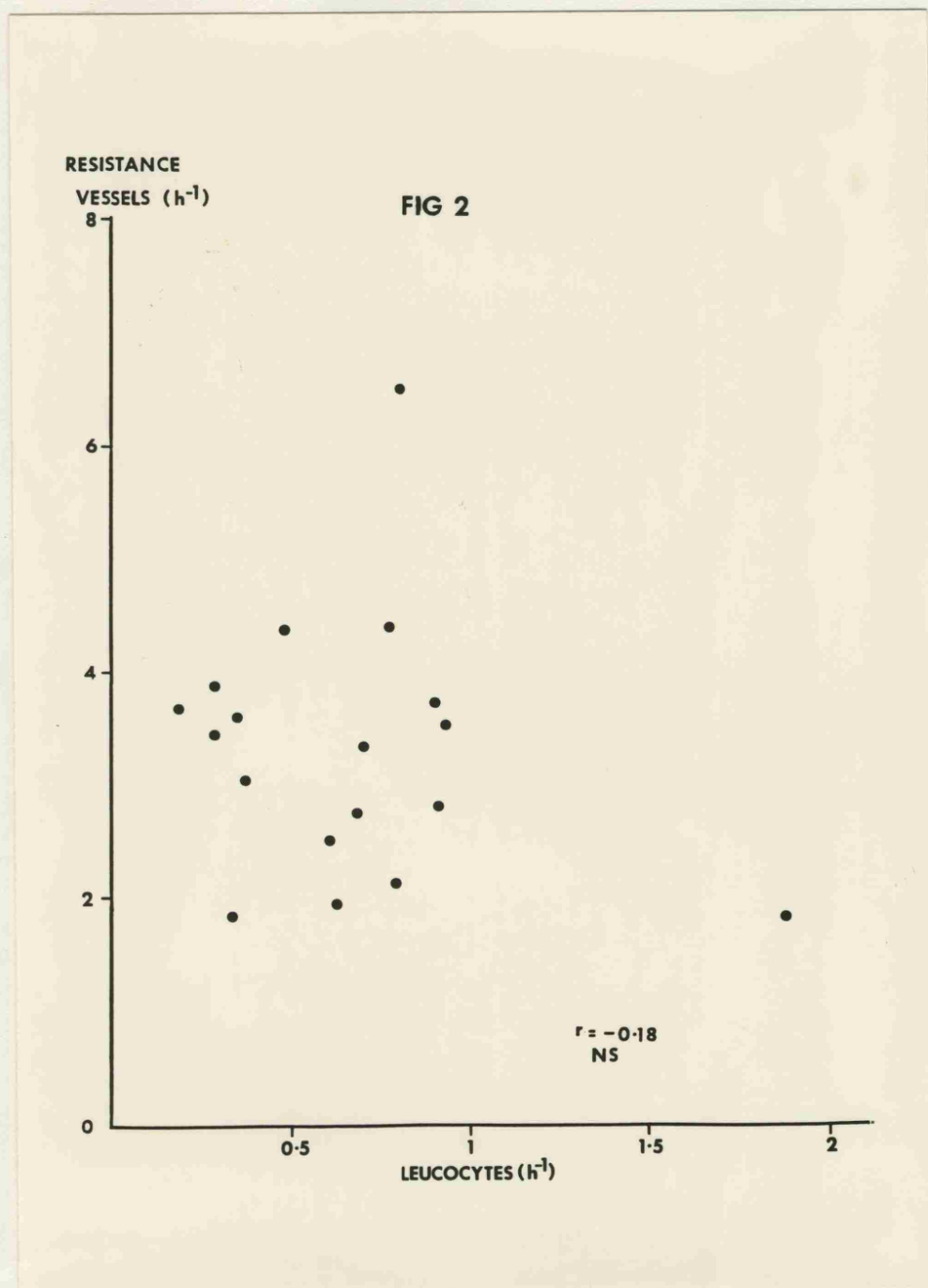


Figure 2: Correlation of ouabain resistant ERC in resistance vessels and leucocytes.

(ERC: Efflux rate constant).

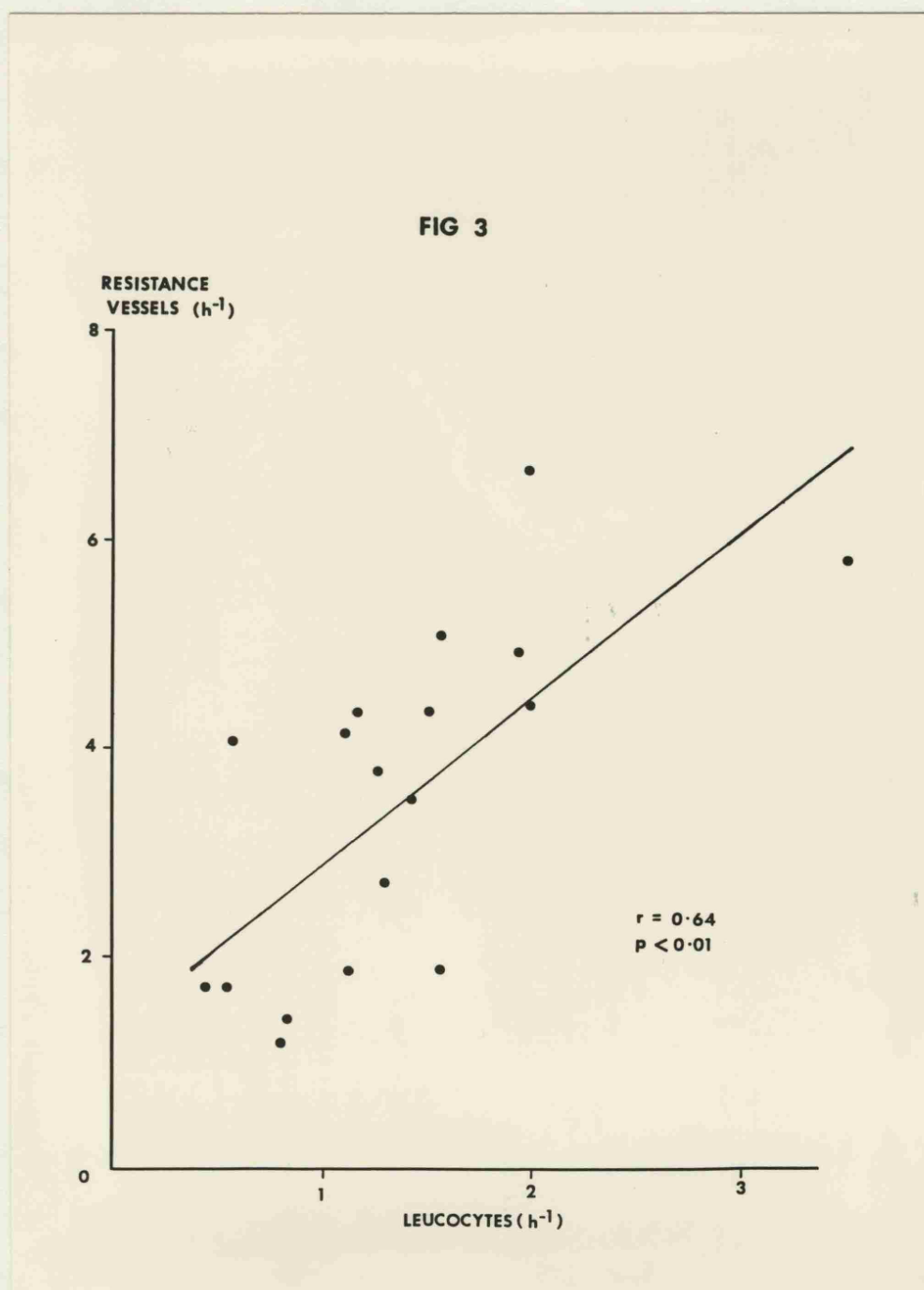


Figure 3: Correlation of ouabain sensitive ERC in resistance vessels and leucocytes.

(ERC: Efflux rate constant).

Chapter 6

Salt Intake and Leucocyte

Sodium Transport

Introduction

The results in the previous Chapter demonstrate that the leucocyte exhibits marked similarities with the resistance blood vessel with regard to transmembrane sodium handling. As a more accessible tissue than blood vessels it was considered a suitable model for more expansive studies of sodium transport in leucocytes. My previous experiments have endeavoured to test whether disturbances in membrane handling of univalent ions are implicated in abnormal blood pressure regulation or merely a loosely associated genetic marker. The hypothesis suggesting the former argument ascribes humoral inhibition of the sodium pump to volume expansion; (de Wardener 1980) a crucial test of the first hypothesis is whether volume expansion will produce reduction in sodium efflux. If, however, the abnormalities described are a manifestation of a genetically determined intrinsic disturbance of the physicochemical structure of the plasma membrane it may also be possible that stimuli which modify ion fluxes may induce a different response in individuals genetically predisposed to hypertension. In order to test these hypotheses further it was decided to investigate the effects of changes in sodium balance on blood pressure and leucocyte sodium transport in two groups of subjects in which differences in leucocyte sodium transport had been described: normotensive first-degree relatives of essential hypertensives and controls.

Subjects

Sixteen healthy normotensive subjects were studied, all with at least one parent hypertensive (in 3 cases both parents were hypertensive). These subjects were compared with fifteen normotensive controls with no family history of hypertension in siblings or parents. All subjects were Caucasian and the groups did not differ

significantly in mean age, weight, plasma renin activity (PRA) or blood pressure (Table 1). Classification of the volunteers was achieved by interview and if there was doubt, parents or siblings were either invited to the Department of Medicine or asked to visit their general practitioner to have their blood pressure measured.

Experimental Protocol

All subjects were assessed by a dietician to estimate the caloric intake of their normal diet. After recruitment volunteers were then asked to provide three complete 24 hour urine collections to allow calculation of their sodium and potassium intakes, and the subjects' age, weight, height and blood pressures were measured and recorded. Venous blood was obtained for leucocyte sodium transport studies, intracellular electrolyte content and PRA. Subjects were then randomized to receive either a high or low sodium intake for 14 days. At the end of the study period volunteers provided a further 24 hour urine collection for electrolyte excretion and blood for leucocyte sodium transport studies, electrolyte content and PRA, as well as having their blood pressure measured. After a washout period of 2 weeks, subjects were switched to the other arm of the diet and were restudied after a further 14 days. High sodium intake was achieved by augmenting normal diet with 15 'slow sodium' tablets (Ciba Labs, Horsham, UK) daily. Each tablet contains 10 mmol Na^+ in a slow release core: the purpose of this regime was to double normal sodium intake. The low salt diet was provided by the diet kitchen at Leicester Royal Infirmary, and all meals were taken there. The aim of the diet was to lower daily sodium intake to 40 mmol; each individual had the caloric intake adjusted to avoid weight loss as a result of reduced calorific value of food.

The study was approved by the Local Ethical Committee and all subjects gave full informed consent.

Methods

Leucocyte sodium transport studies and PRA were measured as outlined above. Urinary electrolyte estimations were performed using a Corning flame photometer and blood pressures were measured using a Hawksley Random Zero Sphygmomanometer.

Statistical Analysis

Within group analysis was performed using student's paired 't' test, comparing data on high and low salt intakes, these being the only two randomized dietary periods. Between group analysis was performed on data on normal diet using the Mann Whitney U test. The pattern of response in the two groups on normal diet and the two salt diets was compared using two way analysis of variance. All results were analysed on a Cyber computer and two way analysis was performed using a BMDP Statistics Package. Results are expressed as mean $\pm$ SEM.

Results

All subjects recruited to the study completed both arms of the protocol and there were no significant differences in baseline characteristics between the two groups on normal diet (Table 1). Taking 'slow sodium' tablets produced no ill-effects but the low salt diet was heavily criticised by most volunteers as being bland and unpleasant. Mean body weight fell significantly on low salt diet when compared to high salt intake in both groups (Tables 3 and 4) and urinary electrolyte excretion and PRA confirmed good compliance and further that both dietary manoeuvres achieved their designed objectives (Tables 3 and 4).

Sodium Efflux Rate Constant, Electrolytes and Efflux Rate

The mean total and ouabain-sensitive efflux rate constants were both depressed in relatives compared to control subjects on normal diet although they did not quite attain statistical significance. Similarly, intracellular sodium and total unidirectional efflux rate for sodium were higher in relatives although not statistically significant (Table 2).

In control subjects changes in salt intake failed to alter efflux rate constant, intracellular sodium or total sodium efflux rate (Table 5).

In the relatives of hypertensive patients, both dietary changes increased mean total leucocyte efflux rate constant (Table 6). There was a significant increase in mean ouabain resistant efflux rate constant on low salt intake compared to high salt intake ($p < 0.05$). Intracellular sodium and potassium did not alter and total sodium efflux rate was not changed on either diet (Table 6).

Using two way analysis of variance it was possible to compare the pattern of response in leucocyte sodium transport in the two groups of subjects during the two dietary study periods. This revealed a significantly different change in mean total leucocyte efflux constant ($p < 0.05$); this reflected the fact that in control subjects neither diet influenced total efflux rate constant, whereas in relatives it was stimulated by both manipulations.

Blood Pressure

In both groups of subjects, supine blood pressure was not different on low compared to high salt intake (Tables 3 and 4). However, both groups showed falls in standing pressures on low salt diet, attaining statistical significance in both systolic ($p < 0.001$) and diastolic ($p < 0.01$) components in controls and in

systolic pressure ($p < 0.01$) in relatives. These postural changes produced no symptoms in any subject.

Table 1 Clinical Characteristics of Relatives of Hypertensive Patients and Control Subjects on Normal Diet

	N	Sex	Age (yrs)	Weight (kg)	B.P. (mmHg) Lying	B.P. (mmHg) Standing	UNa (mmol/ 24 hrs)	UK (mmol/ 24 hrs)	PRA (ng AI ml ⁻¹ h ⁻¹)
Controls	15	M:10 F: 5	26+1.7	70+3.4	126+3.5	117+3.5	149+13	75+4.7	4.29+0.77
					70+4.1	76+2.8			
Relatives	16	M:8 F:8	25+1.4	67+3.1	127+2.9	123+2.5	144+14	66+5.0	4.20+0.54
					67+3.1	77+2.5			
			(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)

(NS - not significant)

Table 2 Mean Efflux Rate Constants, Intracellular Electrolyte Contents and Efflux Rates of Relatives of Hypertensive Patients and Control Subjects on Normal Diet

	Mean Efflux Rate Constant (h^{-1})		Intracellular Electrolyte Content (mmol/kg dry weight)		Sodium Efflux Rate (mmol/kg/h^{-1})
	Total	OR	OS	(Na) _i (K) _i	
Controls	2.01±0.11	0.65±0.07	1.37±0.09	44±2.7 366±8.3	89±8.4
Relatives	1.85±0.08	0.73±0.08	1.12±0.1	52±4.8 372±8.9	97±10
	p=0.07		p=0.063		

(OR - ouabain resistant: OS - ouabain sensitive)

Table 3 Mean Weights, Blood Pressure, Urinary Electrolyte Excretion and PRA in Control Subjects on High and Low Salt Intakes

Control Subjects						
Dietary Period	Wt (kg)	B.P. (mmHg) Lying	Standing	UNa (mmol/ 24 hr)	UK (mmol/ 24 hr)	PRA (ng AI ml ⁻¹ h ⁻¹)
Low Salt	68+3.2***	128+2.5	110+3.3***	39+3.9	70+4.6	7.1+1.4**
		70+3.1	76+3.5*			
High Salt	70+3.3	128+2.7	120+3.7	264+14.0	80+7.1	1.5+0.19
		71+4.0	80+3.2			

(*** p<0.001; ** p<0.01; * p<0.05).

Table 4 Mean Weights, Blood Pressures, Urinary Electrolyte Excretion and PRA in Relatives of Hypertensive

Patients on High and Low Salt IntakesRelative Subjects

Dietary Period	Wt (kg)	B.P. (mmHg) Lying	Standing	UNa (mmol/ 24 hr)	UK (mmol/ 24 hr)	PRA (ng AI ml ⁻¹ h ⁻¹)
Low Salt	66 _± 2.9**	131 _± 2.7	117 _± 1.7**	39 _± 5.3***	60 _± 4.0	7.3 _± 1.8*
		67 _± 2.5	76 _± 2.3			
High Salt	68 _± 2.9	130 _± 3.3	127 _± 3.7	256 _± 32	69 _± 8.1	2.7 _± 0.8
		68 _± 3.2	73 _± 2.8			

(*** p < 0.001; ** p < 0.01; * p < 0.05).

Table 5 Mean Efflux Rate Constants, Intracellular Electrolyte Contents and Efflux Rates of Control Subjects on High and Low Salt Intakes

Dietary Period	Efflux Rate Constant (h^{-1})	Intracellular Electrolyte Content (mmol/kg dry weight)		Sodium Efflux Rate (mmol/kg/h^{-1})
		(Na) _i	(K) _i	
	Total	OR	OS	
Low Salt	1.94 $\pm$ 0.09	0.8 $\pm$ 0.07	1.15 $\pm$ 0.07	103 $\pm$ 11
High Salt	1.94 $\pm$ 0.09	0.7 $\pm$ 0.05	1.3 $\pm$ 0.09	93 $\pm$ 10.4
	(NS)	(NS)	(NS)	(NS)

(OR - ouabain resistant; OS - ouabain sensitive)

Table 6 Mean Efflux Rate Constants, Intracellular Electrolyte Contents and Efflux Rates of Relatives of Hypertensive Patients on High and Low Salt Intakes

Dietary Period	Efflux Rate Constant (hr^{-1})		Intracellular Electrolyte Content (mmol/kg dry weight)		Sodium Efflux Rate (mmol/kg/h^{-1})
	Total	OR	OS	(Na) _i (K) _i	
Low Salt	2.28 $\pm$ 0.11	0.92 $\pm$ 0.09*	1.35 $\pm$ 0.14	46 $\pm$ 3.5 363 $\pm$ 7.0	104 $\pm$ 10.7
High Salt	2.17 $\pm$ 0.15	0.74 $\pm$ 0.08	1.37 $\pm$ 0.12	52 $\pm$ 3.5 357 $\pm$ 10.1	114 $\pm$ 12.6

(* $p < 0.05$).

(OR - ouabain resistant: OS - ouabain sensitive)

Chapter 7

Changes in Leucocyte Sodium Transport in
Normotensive Relatives of Hypertensive Subjects:
Dissociation from Blood Pressure.
Experiments Using Diuretics to Alter Sodium Balance.

Introduction

The work in the preceding chapters has demonstrated abnormalities of sodium handling by the plasma membrane in essential hypertensive patients and their normotensive offspring. If the hypothesis of de Wardener (1980) is correct the manipulation of the sodium pump acutely should be associated with changes in blood pressure. Indeed evidence has been reported in favour of this argument with the finding that the fall in blood pressure provoked by thiazide diuretics is associated with restoration of depressed sodium pump activity and the return of intracellular sodium to normal (Poston et al 1981b; Araoye et al 1978).

With the demonstration of perturbations of sodium handling by normotensive relatives of essential hypertensive patients (Heagerty et al 1982) it has been argued that if such changes reflect the situation in vascular smooth muscle cells, it is unlikely that the abnormalities participate directly in blood pressure elevation. The aim of this experiment was to investigate whether altering sodium balance using diuretics would alter leucocyte sodium transport in the direction predicted by de Wardener and MacGregor (1980) and whether there was any difference in response to the manipulation in subjects genetically at risk of developing hypertension.

Subjects

Fourteen healthy normotensive subjects were studied all with one or more first degree relatives known to have essential hypertension. Nine were men, five were women and the mean age was 28 years. These subjects were compared with 14 normotensive controls with no family history of hypertension. Participants were Caucasian except for one Asian subject in each group; they did not differ significantly in mean age, height, weight, plasma renin activity

(PRA) or blood pressure (Tables 1 and 2). Classification of the volunteers was achieved by an initial interview in which for each subject the blood pressure status of all first degree relatives was documented. If there was doubt, relatives were asked to have their blood pressure checked at the Department of Medicine or by their general practitioner. If doubt remained, the subject was not studied. All blood pressures were measured with a Hawksley Random Zero Sphygmomanometer and full ethical committee approval was granted and informed consent was obtained from all volunteers.

Methods

Standard procedures for carrying out leucocyte sodium transport studies were employed as was the method for measuring PRA. In addition to obtaining blood for efflux rate constant, electrolyte estimations and PRA, additional blood was taken for determination of serum electrolytes, urea, creatinine and urate. Subjects were then given bendrofluazide 5 mg orally once daily starting on the day after the first blood sample. After 7 days a second blood sample was obtained and subjects with and without a family history of hypertension were started in random order. Statistical analysis of efflux rate constants for the two populations was by Mann-Whitney U test for unpaired observations and sign test with binominal distribution for paired observations. Other comparisons were by paired and unpaired students 't' test as appropriate.

Results

Sodium Efflux Rate Constant

Before Diuretic

There was a highly significant reduction in the mean total efflux rate constant for sodium in the relatives compared to control subjects ($p < 0.01$). This was due to a significant depression

in the ouabain sensitive component ($p < 0.02$), the ouabain resistant component was slightly lower in the relatives but did not attain statistical significance (Table 3).

After Diuretic

There was no significant change in the mean total sodium efflux rate constant after diuretic in the control group. Neither ouabain resistant nor ouabain sensitive efflux was altered (Fig 1). However, in the relatives there was a highly significant rise in the total sodium efflux rate constant after administration of the diuretic when compared to before. This was attributable to rises both in glycoside resistant and sensitive pathways, although only the latter attained statistical significance. The effect of the diuretic was to bring the efflux rate constants for the relatives into the normal range, so that after therapy there was no significant difference between the two groups (Table 3).

Intracellular Sodium Content and Sodium Efflux Rate

Intracellular sodium content was not significantly different in the two groups before therapy (49 ± 5.6 mmol/kg dry wt in the relatives vs 48 ± 3.6 mmol/kg dry wt for the control group) and did not alter significantly after 1 week of diuretic therapy (51 ± 3.4 vs 50 ± 4.6 mmol/kg dry wt cells). Sodium efflux rate remained unchanged in the normal subjects before and after therapy (120 ± 8.7 vs 118 ± 13.4 mmol/kg h^{-1}). In the relatives the efflux rate rose significantly after diuretic therapy (93 ± 10.7 vs 125 ± 10.1 mmol/kg h^{-1} , $p < 0.02$).

Plasma Renin Activity

There was no difference in PRA in relatives compared to control subjects before the diuretic (7.3 ± 0.8 vs 6.3 ± 0.7 ng Angiotensin I $ml^{-1} h^{-1}$, $p > 0.2$) after 7 days of taking bendrofluazide both the relatives and control subjects showed a marked similar rise

in PRA, which indirectly indicated good compliance (12.4 ± 1.4 vs 14.6 ± 1.9 Angiotensin I $\text{ml}^{-1} \text{hr}^{-1}$, $p > 0.2$).

Serum Electrolytes, Urea, Creatinine and Urate

Serum sodium did not differ significantly in either group before or after the diuretic (Table 4). Serum potassium was similar in both groups before the diuretic but after 7 days of therapy it fell significantly in the control group ($p < 0.005$). In the relatives group, K^+ also fell after the diuretic but the value just failed to achieve statistical significance ($p = 0.07$). Serum urea and creatinine were not significantly different in the groups before or after the diuretic. The urate levels which were similar before the administration of bendrofluazide rose significantly in both of the groups ($p < 0.05$).

Blood Pressure

There was no significant difference in the mean blood pressure of the two groups before the diuretic nor did it fall significantly in either group after the diuretic (Table 2). Only the change in supine systolic blood pressure in relatives reached statistical significance ($p < 0.05$). There was no significant difference in the change in either systolic or diastolic blood pressure after diuretic treatment in the two groups.

Table 1 Physical Characteristics of Relatives of Hypertensive Patients and Control Subjects (Values are

Expressed as Mean $\pm$ SEM)

	Relatives	Controls
N	14	14
Males	9	10
Females	5	4
Age (yrs)	28 $\pm$ 1.8	28 $\pm$ 2.0
Height (m)	1.7 $\pm$ 0.33	1.72 $\pm$ 0.29
Weight (kg)	66.6 $\pm$ 2.7	67.6 $\pm$ 2.5

Table 2 Blood Pressure (BP) Before and After Diuretic Therapy

Blood Pressure (mmHg)	Relatives		Controls	
	Before	After	Before	After
Supine				
Systolic	124 $\pm$ 3.9	118 $\pm$ 3.8	122 $\pm$ 3.8	121 $\pm$ 1.8
Diastolic	67 $\pm$ 3.6	68 $\pm$ 2.6	68 $\pm$ 2.5	69 $\pm$ 2.9
Standing				
Systolic	123 $\pm$ 4.5	118 $\pm$ 3.3	120 $\pm$ 3.4	116 $\pm$ 1.8
Diastolic	74 $\pm$ 2.8	74 $\pm$ 3.4	78 $\pm$ 2.0	79 $\pm$ 1.9

Table 3 Leucocyte Sodium Efflux Rate Constants Before and After Therapy

Group	Before Diuretic (h^{-1})			After Diuretic (h^{-1})		
	Total	Ouabain Resistant	Ouabain Sensitive	Total	Ouabain Resistant	Ouabain Sensitive
Control Subjects	2.48 ± 0.07	0.89 ± 0.06	1.59 ± 0.06	2.39 ± 0.09	0.89 ± 0.07	1.49 ± 0.1
	$p < 0.001$	NS	$p < 0.02$	NS	NS	NS
Relatives	1.98 ± 0.06	0.71 ± 0.06	1.26 ± 0.1	2.51 ± 0.08	0.88 ± 0.07	1.63 ± 0.1

Table 4 Serum Electrolytes, Urea, Creatinine and Urate Before and After 7 Days of Bendroflumazide Therapy

Serum Concentrations	Controls		Relatives	
	Before	After	Before	After
Sodium (mmol/l)	140±0.3	140±0.4	140±0.5	140±0.5
Potassium (mmol/l)	4.1±0.08	3.83±0.08*	4.1±0.06	3.9±0.09
Urea (mmol/l)	4.2±0.3	4.7±0.35	4.8±0.4	5±0.2
Creatinine (umol/l)	89±4.5	88±3.9	80±4.0	83±3.8
Urate (umol/l)	353±22	393±21*	369±25	409±30*

* p < 0.05

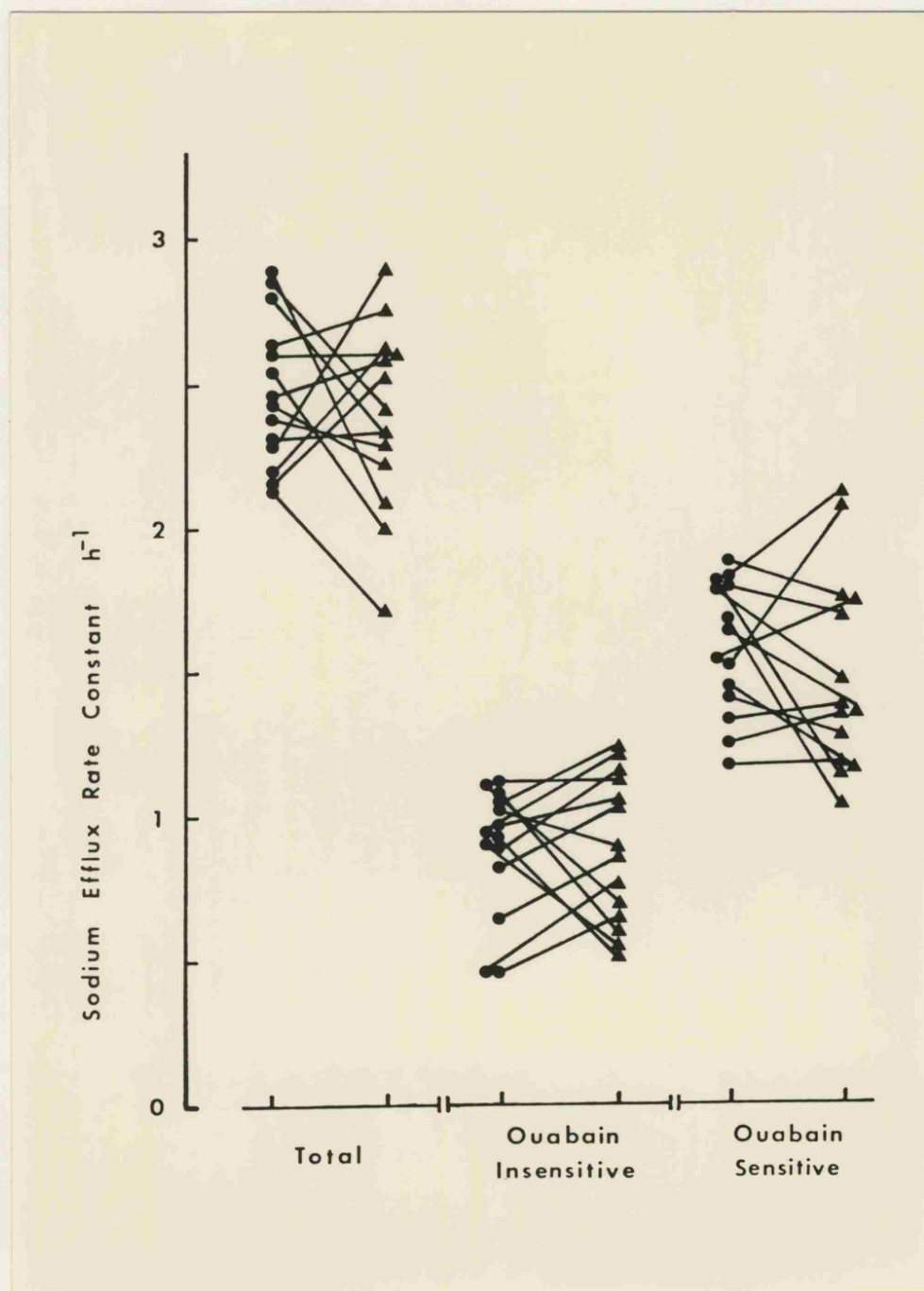


Figure 1: Leucocyte sodium efflux rate constants (h^{-1}) in control subjects before (●) and after (▲) diuretic.

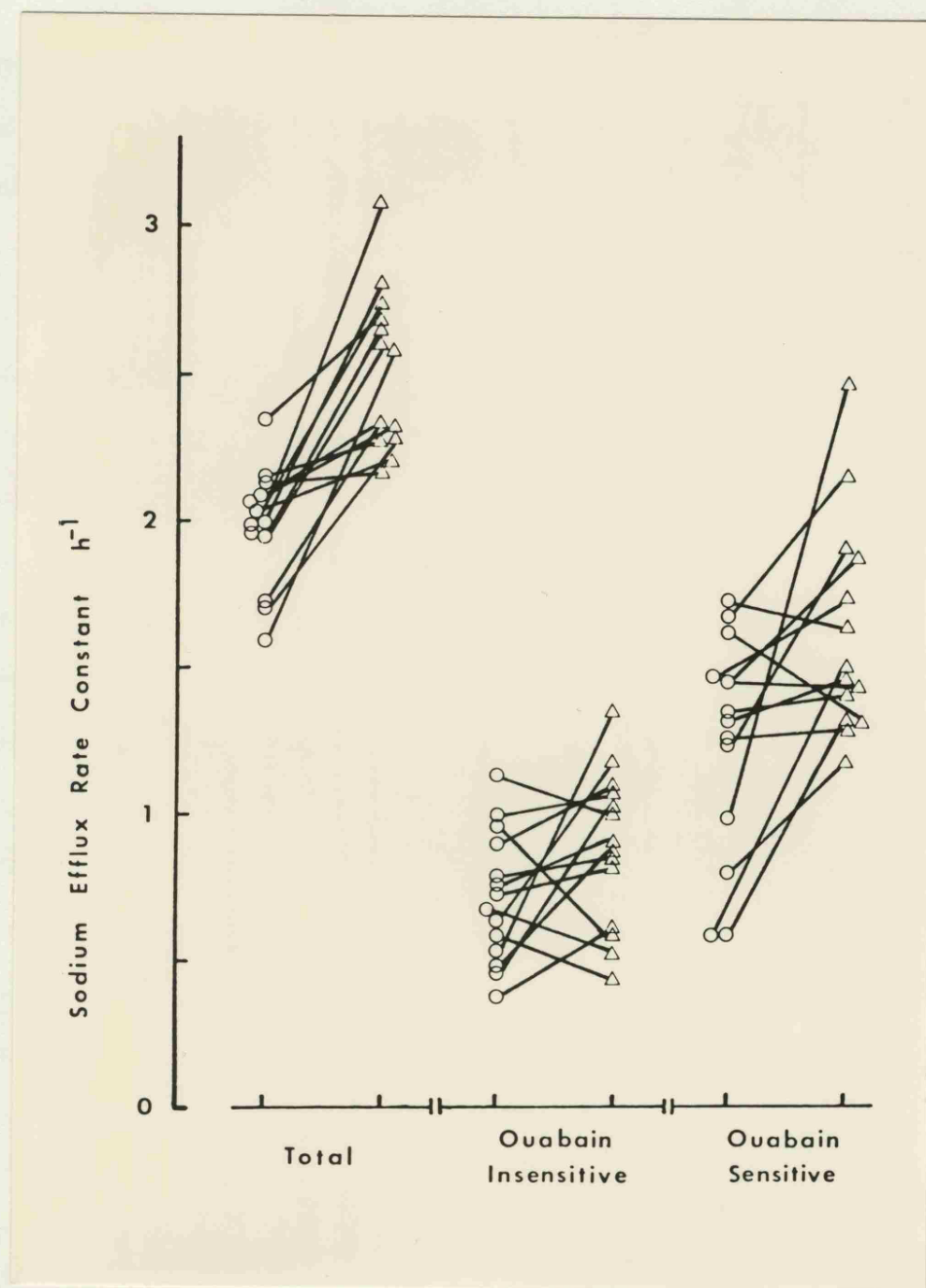


Figure 2: Leucocyte sodium efflux rate constants (h^{-1}) in relatives of hypertensive patients before (○) and after (△) diuretic.

Chapter 8

Discussion of Results

1. Experiments on Leucocyte Sodium Transport in Essential Hypertensive Patients and Their Normotensive Offspring

This is the first full study I have performed in essential hypertension; a previous report (Heagerty et al 1982) demonstrated no significant difference in the rate constants between hypertensive patients and normotensive offspring of hypertensive patients. However, the lack of suitable age-matched controls made an analysis with the hypertensive patients impossible at that time. Nevertheless, the values obtained in this thesis do not differ greatly from those observed in 1982. Previous studies have reported significantly lower total rate constants in essential hypertension (Edmondson et al 1975; Poston et al 1981) and both suggested that the reduction was due solely to lower ouabain sensitive sodium potassium ATPase pump activity. However, beyond this no firm conclusions can be drawn from these two reports with regard to sodium content or absolute sodium movements because in the former study the sodium content was measured at room temperature and in the latter report the sodium concentrations clearly had problems with reproducibility which make interpretation difficult. In these present studies both ouabain resistant and ouabain sensitive pathways were lower and leucocyte sodium was slightly higher in hypertensive patients with absolute sodium efflux rates also being slightly greater than in control subjects. The finding of an abnormality after the disease process has been established still leaves it uncertain whether it is caused by hypertension or causally related to it. Nevertheless, previous similar (although more marked) abnormalities have been cited as evidence for a humoral ouabain-like factor (de Wardener and MacGregor 1980) released in excess as a result of a genetically determined inability of the kidney to excrete sodium adequately. In order

to promote a natriuresis, a factor is secreted which has ouabain-like properties and in addition to increasing renal sodium excretion it inhibits Na^+/K^+ ATPase activity which will raise internal cell sodium content. This inhibits a putative sodium calcium exchange mechanism in smooth muscle cells (Blaustein 1977) and the subsequent elevation of calcium increases smooth muscle reactivity. Certainly the small falls in sodium efflux rate constants noted in these studies of hypertensive patients are consistent with this hypothesis although the differences do not reach statistical significance and are therefore of dubious importance. However, the subsequent experiments demonstrating significantly reduced total efflux rate constant in the normotensive offspring of hypertensive patients argue against this being the case. Only two other studies have been performed on leucocytes from normotensive populations. The first by Gray et al (1984) demonstrated no abnormalities at all in relatives compared to control subjects. The study was well performed and apart from an imbalance of females in the two groups cannot be criticised; I am unable to explain the apparent discrepancy between these data and my own, especially when there is a large measure of agreement in the other areas that this group of workers and myself have both investigated. Chien and Guang-Sheng (1984) have reported reduced ouabain resistant efflux rate constants in leucocytes from normotensive offspring of Chinese hypertensive patients, while ouabain sensitive activity was normal. This latter study is in closer agreement with my own data which showed that the significant fall in total efflux rate constant was due to non-significant falls in both ouabain resistant and ouabain sensitive pathways.

The ouabain resistant efflux rate constant comprises lithium-

sodium countertransport, sodium-sodium cotransport as well as passive permeability to sodium. Both countertransport and cotransport have been found to be abnormal in the normotensive offspring of hypertensive patients (Garay et al 1980; Canessa et al 1982). An hypothesis which invokes a ouabain-like inhibitor is unable to explain these findings.

In summary, the results obtained in hypertensive patients demonstrated a slight reduction in leucocyte ouabain sensitive efflux rate constant and small rise in internal sodium content. The overall effect was a small fall in net sodium efflux via the Na^+/K^+ pump, although this was balanced by a small rise in ouabain resistant efflux. Net flux was thus unaltered and it is difficult to see how these findings can be implicated in the genesis of hypertension. Indeed, similar although not identical results were obtained in a large group of normotensive relatives of hypertensive patients. The fact that these experiments were performed in buffer rather than plasma might suggest that any inhibitor was washed off in preparing the cells. However the method used was similar to that employed by others when the original changes were seen in hypertensive patients. Taken together, these data dissociate sodium movements from the day to day cell changes that influence blood pressure and provide no support for a tightly bound humoral factor in the blood of hypertensive subjects, which inhibits the sodium pump.

2. Experiments Correlating Sodium Fluxes in Human Resistance Vessels and Leucocytes

The demonstration of strong correlations in total sodium efflux rate constants and ouabain sensitive efflux rate constants in leucocytes and blood vessels implies that to a certain extent the extrapolation of results on sodium transport in leucocytes to what occurs in blood

vessels is justified. Pathogenetic importance has been attached to changes in the flux of sodium across the vascular smooth muscle cell membranes and these data provide information on the handling of sodium by human resistance vessels for the first time. In addition these results demonstrate that peripheral blood leucocytes behave in some respects in a similar manner to vascular tissue. The total extrusion of sodium from resistance vessels proceeded at a faster rate in vascular tissue: total efflux rate constants were over three times higher than in leucocytes; nevertheless, there was a significant correlation between the total efflux rate constants in the two tissues. However, the ouabain resistant rate constants were not associated, whereas active sodium pumping as assessed by the ouabain sensitive efflux rate constant was very significantly correlated in blood vessels and white blood cells. The experiments in the two tissues were performed together and the methods used were exactly as previously described. In particular, the leucocyte measurements employed the methods used to demonstrate the abnormalities documented in the Chapter dealing with studies in essential hypertensive patients and their offspring.

The failure to find a correlation in ouabain resistant efflux rate constants between the two tissues deserves comment. This component of sodium efflux is made up of a combination of processes including passive sodium movement as well as a number of other fluxes such as sodium:sodium cotransport and sodium-lithium countertransport. In blood cells the cellular extrusion of sodium is straightforward. However, in resistance vessels such measurements are complicated by the extracellular space. Thus, it is possible that the release of sodium from extracellular sites said to be abolished by cooling might have introduced an added factor when considering ouabain-

resistant fluxes which confounds any possible correlation.

The effects of drugs used during induction of anaesthesia or the anaesthetic gases themselves are unlikely to have influenced sodium fluxes since both leucocytes and blood vessels were thoroughly washed before experimentation began. If any effect of anaesthetic had occurred it is likely to have diminished rather than enhanced correlations.

The experiments here do not provide information on internal sodium content or absolute flux rates in either tissue. The coefficients of variation for internal sodium measurement in both tissues make interpretation of results hazardous. As the relationship between intracellular sodium concentration and its efflux by means of the sodium pump is linear in the leucocyte up to concentrations that are more than twice the normal values (Hilton 1986), the first order rate constant is the most satisfactory way of providing information on this mechanism of sodium extrusion at least. The significant positive correlations between rate constants in leucocytes and blood vessels are therefore good evidence that the leucocyte at least reflects in part what occurs in blood vessels. In this regard these data lend support to the use of the leucocyte as a way of examining sodium transport processes in hypertension.

3. Changes in Sodium Intake and Leucocyte Sodium Transport

The manipulations of dietary salt intake were designed to test whether plasma volume expansion and contraction led to alterations in the levels of a putative sodium transport inhibitor. The theory of de Wardener and MacGregor postulates that essential hypertension is a disease of volume overload: the kidney is subjected to an assumed excessive salt intake and in genetically susceptible subjects it is unable to excrete the load sufficiently and plasma volume expands. This leads to the secretion of a ouabain-like factor which promotes

a natriuresis and, in addition, depresses Na^+/K^+ ATPase activity which raises pressure. The association between salt intake and essential hypertension has been detailed in the introduction to this thesis and remains tenuous. Nevertheless, more fundamental criticisms can be levelled at this aspect of the hypothesis, the principal one being that there is no evidence for fluid overload in essential hypertension. In fact, as outlined above, the opposite appears to be the case, certainly in the early phase of this disease (Birkenhager and de Leeuw 1984). Nevertheless, the crucial experiment was to vary sodium intake and investigate the effects on sodium transport and blood pressure; in particular, the experiment performed addressed the question of whether inhibition of leucocyte pumping would be induced by sodium loading. In neither group of experimental subjects was this observed; nor did altered sodium balance change intracellular sodium. Dietary sodium intake was altered by a factor of more than six-fold and this was sufficient to produce significant changes in blood pressure. It is unlikely therefore that inhibition of sodium transport over the period of study was missed as a result of inadequate dietary change. The results are in contrast to those of Ambrosioni et al (1982) who demonstrated a reduction in intralymphocytic sodium associated with a reduced pressor response to stress in salt restricted subjects. However, leucocyte ion fluxes were not measured in this study. A further possibility is that the effects any humoral factors might have been abolished by washing and performing the experiments in buffered tissue culture medium; the results of such experiments in physiological media may effect differences at the time of study and may not be truly representative of what occurs in vivo. However, the method employed was the same as that used when abnormalities of leucocyte sodium transport in the offspring

of hypertensives were demonstrated outlined in previous chapters and similar to that used by other groups to demonstrate abnormalities in leucocyte sodium fluxes in hypertension. If these abnormalities had been produced by changes in sodium balance the present studies would therefore have demonstrated an effect.

Despite its possible relevance to the pathophysiology of hypertension, there have been comparatively few studies of cell membrane sodium transport during dietary salt restriction and loading. In the normal rat thymocyte, very severe salt restriction reduced the ouabain sensitive and total sodium efflux rate constants (Bradlaugh et al 1984). In man, studies have so far been confined to the erythrocyte. Morgan et al (1981) reported a significant reduction in erythrocyte sodium efflux rate constant with salt restriction in 8 hypertensive patients, whilst Cooper and co-workers (1984) could detect no change in intracellular sodium or lithium-sodium countertransport with dietary sodium reduction. Weissberg et al (1984) produced no significant changes in sodium efflux or intracellular sodium in normal volunteers with high and low salt diets. Consistent with the present observations, Gudmundsson (1984) reported an increase in erythrocytic sodium efflux rate constant with salt loading. The most recent study is by Stokes (1986). He investigated severe salt restriction (20 mmol/day) in normotensive and hypertensive subjects. Blood pressure fell in the hypertensive group and failed to change in the controls. No change in sodium pump activity was observed in either group. There is, therefore, no support for the suggestion that sodium loading is responsible for the inhibition of the sodium pump which has been observed in the leucocytes of hypertensive patients and their offspring. In this diet study, low salt intake gave rise to a significant increase in ouabain resistant sodium fluxes, reflecting

an increase in membrane permeability and non-ATPase mediated pathways in relatives. Just as in the diuretic study however, there was a significant difference in the pattern of response of total sodium efflux rate constants to changes in salt intake between relatives and controls: the rate constant was unaltered in control subjects whilst it rose in subjects with a family history of hypertension. This could reflect either differences in the responsiveness of the plasma membrane or alterations in a tightly bound circulating factor which controls ionic fluxes and which is not removed by the in vitro procedures. The present study cannot differentiate between the two and the interpretation of both the dietary salt experiment and the diuretic experiments remains a matter of speculation. Nevertheless, the pattern of response predicted by an hypothesis postulating changes in concentration of a humoral inhibitor in response to changes in plasma volume was not observed. In fact, two facts emerged: there were different responses seen in subjects with a family history of hypertension compared to controls and the differences are unlikely to be attributable to excess glycoside-like activity.

4. Diuretics and Sodium Transport

This experiment was performed to try and change sodium pump activity by diuretic administration; thiazide diuretics were used as agents which undoubtedly modify sodium balance but in a manner which differs from dietary manipulation. In addition there is the possibility that diuretics influence blood pressure independently of induced changes in sodium balance. It has previously been shown that the use of such drugs to lower blood pressure was associated with the restoration of depressed sodium pump activity and the return of intracellular sodium to normal (Poston et al 1981(b); Araoye et al (1978)).

The subjects studied were normotensive with and without a family history of hypertension, the latter group having already been shown to have abnormal leucocyte membrane sodium transport mechanisms. My results suggest a similar stimulation of sodium transport in normotensive offspring of essential hypertensive patients to that seen in studies of hypertensives (Poston et al 1981(b), Araoye et al 1978). Both groups of subjects showed falls in blood pressure and in the relatives the fall in supine systolic pressure reached significance. However, there was no significant difference between the mean blood pressure in either group before or after the diuretic and diastolic pressures actually rose. There was a dramatic rise in sodium pump activity in relatives, however this was associated with a slight rise in diastolic blood pressure although systolic blood pressure fell. The response of the control group showed a distinct difference since a slight reduction in sodium pump activity occurred, although blood pressure showed a small, non-significant fall. There is little correlation therefore between leucocyte sodium pumping and blood pressure in this study.

The mode of action of the diuretic on membrane sodium transport is unclear. The use of thiazide diuretics in the treatment of essential hypertension is associated with an acute decrease in plasma volume and a natriuresis (Freis et al 1958); the natriuresis appears to disappear with chronic administration (Bing et al 1979). When such diuretics are administered to normotensive subjects, the acute natriuresis, weight loss and decrease in plasma volume are similar in magnitude, although blood pressure is not affected (Freis et al 1960). During the seven days of this study therefore, it is likely that the subjects would be undergoing a natriuresis. If the humoral inhibitor hypothesis of de Wardener and MacGregor

(1980) is correct, the diuretic should correct the volume expansion postulated to occur early in the course of hypertension and reduce the levels of the ouabain-like factor in the blood and hence sodium pump activity should increase. In the normotensive offspring of hypertensive patients sodium pump activity was indeed enhanced, although mean blood pressure did not fall but in the control subjects no such effect was observed on sodium transport and again blood pressure was not affected. The antihypertensive effects of thiazide diuretics may be attributable in the chronic phase to actions other than those on sodium balance and even in this short term experiment membrane sodium transport characteristics are dissociated from alterations in blood pressure. The effects on sodium transport in my experiments and in hypertensive patients (Poston et al 1981(b)) could be explained in terms other than implicating raised or depressed levels of a humoral pump inhibitor. For example, thiazide diuretics increase plasma cholesterol and particularly LDL-cholesterol and VLDL-cholesterol (Weidmann et al 1983). Changes in cholesterol concentrations are known to alter active sodium pumping (Cooper 1977), an indirect effect on the plasma membrane may well have more relevance to what is occurring in hypertension and will be discussed further below.

Chapter 9

General Discussion and Conclusions

The results of the experiments performed demonstrated only small changes in membrane sodium handling in leucocytes of hypertensive patients which did not reach statistical significance and similar significant changes in the normotensive offspring of hypertensive patients were observed. Both total and ouabain sensitive sodium fluxes were correlated in the leucocyte and the omental resistance vessel suggesting that background variability in these tissues reflects a common property of their cell membranes. In view of widely accepted theories that changes in these transport processes are attributable to changes in sodium balance, experiments were performed altering salt intake. Extreme changes in dietary salt intake produced only small changes in blood pressure and the pattern of response of sodium transport was different when comparing relatives of hypertensive patients to matched control subjects. Attempts to alter sodium transport using thiazide diuretics once again revealed different responses in offspring compared to control subjects but no changes in mean blood pressure.

The principal purpose of the experiments described above was to examine an hypothesis; this was expounded by de Wardener and MacGregor (1980) and postulated that essential hypertension was a disease caused by the kidney genetically incapable of excreting excess sodium. The result of this phenomenon is an expansion of the blood volume leading to the excess secretion of a humoral factor in an attempt to correct this defect by promoting a natriuresis. The factor is thought to have ouabain-like action and therefore inhibits Na^+/K^+ ATPase activity in plasma membranes. The consequent rise in intracellular sodium then inhibits sodium:calcium exchange mechanisms functioning in the plasma membrane. The net result will be a rise in intracellular calcium and thus an increase in

vascular reactivity with blood pressure thereby increasing. The results of the experiments performed above would fail to substantiate this theory. There were falls seen in sodium pump activity in subjects with essential hypertension but they were small and of doubtful importance. Moreover small falls have been observed in ouabain resistant fluxes (Swales 1982) which cannot be readily explained by this hypothesis. Furthermore, significant changes have been demonstrated in the offspring of hypertensive patients at a time when their blood pressure is normal. These membrane abnormalities have effectively been dissociated from cellular abnormalities intimately involved with minute to minute cellular control of blood pressure. The failure of an hypothesis to stand up to testing would normally lead to its abandonment. Frequently however, ad hoc modifications of the hypothesis are made to avoid relinquishing it entirely, although such an approach is of doubtful scientific validity (Popper 1977). The proponents of a circulating sodium pump inhibitor moved to explain findings in normotensive populations by arguing that compensatory mechanisms are acting to keep the blood pressure from rising at an early age but are eventually overcome as time elapses. No such time lag was initially envisaged in the first statements of this inhibitor theory and by introducing such modifications the hypothesis is nearly removed from the realms of scientific testability. The initial hypothesis of Blaustein (1977) upon which rested much of the subsequent theory of de Wardener and MacGregor, made no allowance for a delay in the rise of pressure. In the sequence of events envisaged by Blaustein, a reduction in Na^+/K^+ ATPase pump activity would raise internal sodium and inhibit sodium-calcium exchange mechanisms immediately. This would increase internal calcium concentrations and vascular reactivity. The time

interval allowed from the reduction of sodium pump activity to the increase in pressure was hours and certainly not years. The modification of the hypothesis of de Wardener and MacGregor to allow for compensatory mechanisms to explain changes in normotensive populations is most persuasive. Nevertheless, there are other problems with the assumptions underlying Blaustein's theory. The bedrock upon which Blaustein based his beliefs was the existence of an intimate interaction between transmembrane sodium and calcium movements in vascular smooth muscle cells. The proposal that a mechanism exists in the cell membrane that can transport Na ions in exchange for Ca ions was first made for cardiac muscle cells by Reuter and Seitz (1968) and it is in this tissue and in squid giant axon where much of the work on this system has been performed. Indeed, in the heart, this exchange mechanism may have a significant role not only in determining contractility but under some conditions it may also effect the membrane potential and the shape of the cardiac action potential. The evidence for such a system being present or of physiological importance in vascular smooth muscle cells is less clear. Indeed its existence in this tissue was first suggested by Bohr et al (1969). Recent studies have confirmed that this process does exist in smooth muscle cell membrane (Brading and Lategan 1985) but elaborate experiments by Van Breemen et al (1981) would suggest that its role is minimal except during extreme unphysiological alterations of external sodium concentrations. Indeed recent work has made it clear that even if a Na:Ca exchange exists in vascular smooth muscle, the clear involvement of sodium ions in the generation of tension in many cases can be explained by the operation of mechanisms other than Na:Ca exchange (Brading and Lategan 1985). Other studies employing calcium antagonists

in man have failed to show an influence upon sodium transport (Heagerty et al 1983).

The controversy can be moved back two further steps by considering whether reduction of sodium pump activity can cause vascular contraction. The Na^+/K^+ pump is influenced by internal cellular sodium content, external potassium concentration and a variety of hormones and inhibited by cardiac glycosides. The experiments of Dock and Tainter (1930) and Ross et al (1960) have demonstrated that acute infusions of glycosides raise total peripheral resistance. Part of this is due to increased noradrenaline release (Gillis and Quest 1980) and part due to increased myogenic tone (Ross et al 1960). The elaborate experiments of Robinson et al (1983) demonstrated direct effects on the vascular bed which were sustained for 60 minutes, but whether long term inhibition would cause sustained vasoconstriction is not clear. Similarly, stimulating the sodium pump with potassium causes vasodilation which is probably transient (Brace 1974). These results on vascular beds have now been developed further by direct investigation of resistance vessels (Mulvany et al 1982a,b). Ouabain caused myogenic contraction of rat aorta and tail artery, but there was no long lasting effect on 150 μm mesenteric and 150 μm femoral resistance vessels. In the mesenteric resistance vessels ouabain increased the internal sodium more than four-fold and this was associated with membrane depolarization from -54 mV to -30 mV after 3 hours. Thus, in blood vessels known to be involved in controlling peripheral resistance, inhibition of the sodium pump caused negligible changes. These considerations allied to the increasing evidence cited in previous Chapters which casts doubt on whether essential hypertensive patients are volume expanded make it difficult for an inhibitor hypothesis to be retained.

Indeed, the experiment dealing with salt-loading once again failed to produce sodium pump inhibition, which was only seen with extreme salt depletion.

An alternative explanation for all these findings has been postulated by Swales (1982) and Postnov and Orlov (1984). All these workers have suggested that all the perturbations of membrane sodium handling including disordered countertransport and cotransport systems observed in hypertension can be explained by a genetically acquired abnormality of the physicochemical structure of the plasma membrane. The slight reduction in sodium efflux rates noted in my experiments, for example, now lose their postulated primary role in generating hypertension and are relegated to being loosely associated genetic markers of what remains a disease with multifactorial origins. Moreover, the abnormalities of calcium handling in hypertension are also explained: decreased calcium binding to the outer surface of cell membranes is reported (Gulak et al 1979) and changes in calcium binding to the inner aspect of the membrane and in calmodulin-dependent calcium transport (Postnov et al 1979, Orlov et al 1983) are also observed. The attraction of this hypothesis is that it explains the discovery of abnormalities of cell membrane function in cells and tissues that are not directly involved in blood pressure generation, the genetic component implying a global disturbance and thus changes reported in blood cells (Swales 1982), hepatocytes, synaptosomes (Devynck et al 1981), and neurones from the central nervous system (Devynck et al 1981, Devynck et al 1982, Kravtsov et al 1982) are to be expected. The cell membrane may be regarded as a sea of lipid with islands of protein, or a matrix of protein with lakes of lipid. Much of the lipid is in the form of a bilamellar leaflet with hydrophilic portions that face the aqueous environment

on either side and hydrophobic portions within the core of the bilayer. The organisation of the membrane provides the potential for membrane lipids to modify membrane function. Indeed as knowledge of the structural complexity of cell membranes has grown, it has become apparent that the composition and fluidity of membrane lipids must be critically controlled to allow cell growth and function. It would seem reasonable therefore to site the abnormality of the cell membrane in hypertension in the lipid fraction if it is to be held responsible for widespread perturbations in cell membrane activity. For example, the metabolically active transport systems reside in the phospholipid bilayer of the cell membrane. The composition of the bilayer is not rigid and changes in its chemical configuration readily occur for example, when catecholamines bind to adrenoceptors in a target tissue (Roth and Grunfeld 1981). Moreover, alterations in the proportion of unsaturated to saturated fatty acids in the acyl side-chains of membrane phospholipids have been shown to influence membrane fluidity, transport of ions and Na^+/K^+ ATPase activity (Kimelberg 1975; Cooper 1977; Grisham and Barnett 1973). As mentioned briefly above, the difference in response to 7 days diuretic therapy in sodium transport in normotensive offspring of hypertensives and controls may be explainable in terms of a genetically different lipid structure in the membrane affected in different ways by the changes in cholesterol known to occur after the ingestion of thiazide diuretics. Similarly the changes in salt intake particularly the low sodium diet could only be attained by major dietary modifications. Whilst some changes in sodium transport seen in this study may be the inherent consequences of alterations in sodium balance, it is also possible that the changes in fat intake on low salt diet may have played a role. Likewise

the elevation of catecholamine levels as a result of sodium restriction may be relevant (Gordon et al 1967, Kelsch et al 1971). Support for an abnormality in lipid structure is provided by a number of results. In essential hypertension cell membrane fluidity is reduced (Orlov and Postnov 1982), a finding confirmed in SHR (Montenay-Garestier et al 1981) and in addition sialic acid levels have been found to be raised in hypertension (Reznikova et al 1984). The adaptive processes that control membrane fluidity and the pathological states that ensue when fluidity is abnormal suggest that the fluid properties of membrane lipids are important for cell function. For example, the transport of ions and nutrients is a specialized membrane function. A direct relation between transport and membrane fluidity has been demonstrated in red cells (Cooper 1977). Furthermore, sodium-lithium countertransport, lithium-potassium cotransport and frusemide insensitive lithium efflux into magnesium chloride have all been shown to be correlated with plasma triglyceride levels and high density lipoprotein cholesterol levels (Hunt et al 1986). In the same study higher levels for the three membrane transport systems were found in hypertensive patients and their offspring. The association of all three cation transport systems with blood lipids as well as with hypertension implies the existence of a general relationship between blood lipids, membrane cation transport and blood pressure. In addition to these findings Levy and co-workers (1983) examined the temperature dependence of lithium-sodium countertransport in erythrocytes from normal and hypertensive patients. Arrhenius plots showed a change in slope indicating a difference in membrane fluidity, at 30 °C in normal subjects whereas the corresponding point for 75% of hypertensives was about 20 °C. The studies of Hunt et al (1986) and Adragna et al (1985)

provide evidence from cross-sectional and intervention approaches that plasma triglycerides and HDL levels can influence cellular ion transport. The experiments of Hunt also suggests that the cells of hypertensive patients and their offspring respond differently compared to control cells when faced with the same lipid background and together with those of Levy et al (1983) support the theory that there is an abnormality of the physicochemical structure of the cell membrane and that many of the disturbances of electrolyte transport are merely markers of the basic defect.

In conclusion, therefore, the data presented in this thesis suggest that inhibition of the leucocyte pump is not a consequence of volume expansion. There are significant differences in the effects of changes in sodium balance and diuretics on leucocyte ion transport in individuals with a family history of hypertension and in control subjects. Similarities between ion transport in the leucocyte and resistance vessel suggest that the differences may be shared by these two tissues. The finding of abnormalities in subjects genetically at risk of developing hypertension at a time when they were normotensive suggests that the disturbances of sodium transport observed are loosely associated markers rather than intimately concerned with blood pressure regulation. The lack of supportive evidence for an inhibitor hypothesis suggests that the abnormalities demonstrated are more likely to be manifestations of a more fundamental defect in membrane structure. This may well reside in the phospholipid component of the plasma membrane, and future studies of membrane lipid metabolism may provide exciting insights into the aetiology of essential hypertension.

Appendix I

Apparatus Used to Measure Leucocyte Sodium Efflux Rates

Temperature variable shaking water bath	Grant Instruments, Cambridge, UK.
Sterilin containers	Sterilin, Hampshire, UK.
Pasteur suction pipettes	Bilbase, Daventry, Warwickshire, UK.
12 ml conical plastic tubes	Sarstedt, West Germany.
Pipettes (1 ml, 5 ml) 0.1 ml	Gilson Anachem Ltd., Luton, UK.
Reagent 1 ml test-tubes (no. 72)	Sarstedt, West Germany.
Vacutainers (10 ml with lithium heparin anticoagulant)	Becton Dickinson, Rutherford, New Jersey, USA.
Syringe needles 19 guage	Becton Dickinson, Rutherford, New Jersey, USA.
Aluminium foil	Denomaid, UK.
Surgical gloves	Johnson and Johnson, UK.
Plasmagel	Uniscience, Cambridge, UK.
Medium 199 with hanks salts	Gibco, Paisley, Scotland, UK.
²² NaCl	Amersham International, UK.
Methylated spirit	Pharmacy, Leicester Royal Infirmary.
Chillspin Centrifuge	MSE Instruments, Fisons, Crawley, UK.
Coolspin centrifuge	MSE Instruments, Fisons, Crawley, UK.
Weighing balances	
Mettler ME22	Fisons, Gallencamp,

Loughborough, UK.

Mettler BE22

Stop-Clock

English Clock Systems, UK.

Gamma counter

Hewlett-Packard Ltd, UK.

Oven

RW Jennins Scientific,

Notts, UK.

Earles Buffer (x10)

	gm/l
NaCl	68
KCl	4.4
CaCl ₂	2.7
MgSO ₄ ·7H ₂ O	2.0
NaHPO ₄ ·2H ₂ O	1.5
Glucose	10

Medium 199 (Gibco) (x1)

	mg/l
CaCl ₂	140
Fe (NO ₃) ₃ ·9H ₂ O	0.72
KCl	400
MgSO ₄ ·7H ₂ O	200
NaCl	8000
NaHCO ₃	350
NaHPO ₄ ·7H ₂ O	90

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Studies of Leucocyte Sodium Transport in Essential Hypertension

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ABSTRACT

A method was set up and validated to allow the study of sodium efflux in human peripheral blood leucocytes. In view of a postulated association between dietary salt ingestion and the genesis of essential hypertension, movements of sodium were studied across the plasma membranes of leucocytes in hypertensive patients and their normotensive offspring. In hypertensive patients there were small reductions in ouabain resistant and ouabain sensitive efflux rate constants for sodium and a slightly elevated intraleucocytic sodium content. Absolute sodium efflux rates were therefore unchanged compared to control subjects. In the offspring of hypertensive patients, a similar pattern was observed at a time when these subjects had normal blood pressure. In a subsequent series of experiments strong correlations were demonstrated between sodium efflux rate constants in leucocytes and human resistance blood vessels, and in the light of this finding the experiments using leucocytes were extended. In order to test the hypothesis that there is a humoral factor in hypertension that promotes raised blood pressure by inhibition of the Na^+/K^+ ATPase pump, two experiments were performed: one altered sodium balance by the use of diuretics. In control subjects sodium transport and blood pressure were unaffected, and in normotensive offspring of hypertensives sodium pump activity was stimulated with no change in blood pressure. In the other experiment, similar groups of subjects were exposed to extremes of sodium intake. There were small changes in leucocyte sodium efflux, and a different pattern of response was observed between control subjects and the normotensive offspring of essential hypertensives. In neither of these two experiments

were the results compatible with the presence of a circulating sodium pump inhibitor.

The existence of abnormalities of transmembrane sodium handling in individuals with a family history of raised blood pressure, at a time when their blood pressure is normal, dissociates these disturbances from the cellular processes that intimately control blood pressure. In addition manoeuvres designed to expand or contract plasma volume by manipulating sodium balance produced changes in sodium efflux incompatible with the promotion of a humoral sodium transport inhibitor. The changes obtained were more compatible with the presence of a disturbance of a genetically predetermined perturbation in the physico-chemical structure and function of the cell membrane of which these findings are but one manifestation.