UNIVERSITY OF GHANA MEDICAL SCHOOL

DEPARTMENT OF PHYSIOLOGY

DETERMINANTS OF ARTERIAL BLOOD PRESSURE IN URBAN GHANAIANS: PLASMA RENIN ACTIVITY AND SODIUM BALANCE

BY

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DETERMINANTS OF ARTERIAL BLOOD PRESSURE IN URBAN GHANAIANS: PLASMA RENIN ACTIVITY AND SODIUM BALANCE

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BY

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DECLARATION

I, Paul Armah Aryee, hereby declare that this thesis is the result of work done by myself under supervision. Except for literature cited, which served as sources of information, the work is in no way a reproduction, in part or in whole, of any work ever presented for the award of a degree.

Signature:

.....

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.....

REV. DR. I. F. A. HESSE
(Supervisor)

DEDICATION

1. **TO THE GLORY OF GOD**

2. **TO MY WIFE : ANNIE**

   AND

   MY CHILDREN : PAULA
   VANESSA.
   MARCELLIN

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ABSTRACT

In the tropics, prevailing weather conditions exert a tremendous influence on fluid and electrolyte homeostasis of residents. For the tropical resident, who may have evolved an adaptive strategy to conserve sodium and water by renal and hormonal mechanisms, increases in sodium intake may cause changes that would, in the long term, lead to an increase in the arterial blood pressure.

In this study, three serial protocols were followed to elucidate the significant role of sodium balance and the regulatory renin-angiotensin system in determining the arterial blood pressure of urban resident Ghanaians, and as a basis to explain the recent observed increase in the prevalence of hypertension in Ghana.

The first series of the investigation showed a rising trend in the arterial blood pressure with advancing age for 31 randomly selected subjects; a phenomenon associated with populations on a high salt diet. In the second series, low levels of plasma renin activity (mean = 0.71 ± 0.13 pmol Ang. I/ml/hr) were obtained in 10 subjects (8 normotensive and 2 hypertensive). Such low levels of plasma renin activity, signifying a suppressed renin-angiotensin system, is also associated with a high salt intake. Finally, the last series on 11 subjects, who were fed *ad lib,* revealed a high intake of sodium (mean = 295.2 ± 9.0 mmol) at a threshold of 300 mmol above which the subjects could not effectively excrete any extra sodium load.

The significance of such findings, albeit using different subject groups and a small sample population, is that sodium homeostasis can be considered as a major determinant of arterial blood pressure of tropical residents. Given the present recognition of sodium (salt) as an environmental stressor, and considering its ready-availability, accessibility and affordabilty, high intakes, as observed in this study, could lead to elevation of the blood pressure. This may set the stage for explaining the recent observations regarding the increase in prevalence of hypertension in Ghana.

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LIST OF ABBREVIATIONS AND ACRONYMS

AAS	**Atomic adsorption spectrophotometer**
ABP	**Arterial blood pressure**
ACE	**Angiotensin converting enzyme**
ACTH	**Adreno-corticotropic hormone**
ad lib	**Ad libitum**
Ang. I	**Angiotensin I**
Ang. II	**Angiotensin II**
ANOVA	**Analysis of variance**
ANP	**Atrial natriuretic peptide**
AOAC	**Association of Applied Chemistry**
BMI	**Body mass index**
DBP	**Diastolic blood pressure**
ECF	**Extracellular fluid**
GFR	**Glomerular filtration rate**
GSB	**Ghana Standards Board**
IAEA	**International Atomic Energy Agency**
MAP	**Mean arterial pressure**
mmol	**Millimoles**
Na^+	**Sodium ion**
Na-EDTA	**Sodium, ethylenediaminetetraacetic acid**
pH	**Negative logarithm of the H^+ concentration of a solution**
PMS	**Phenylmethylsulphonyl fluoride**
PRA	**Plasma renin activity**
PUL	**Pulse or heart rate**
RAS	**Renin-Angiotensin System**
RIA	**Radio-immunoassay**
SBP	**Systolic blood pressure**
WHO	**World Health Organisation**

CHAPTER ONE

INTRODUCTION AND LTERATURE REVIEW

1.1 General Introduction.

For most part of the year, weather conditions in Ghana are conducive for sweating (Badoe, 1968). The prevailing hot and humid climate, certainly, has a tremendous effect on fluid and electrolyte homeostasis. There is a continuous tendency for individuals to become dehydrated, because large fluid losses occur from the respiratory and cutaneous routes as a means of heat regulation. Such fluid losses may lead to alterations in the extracellular fluid (ECF) and, specifically, the intravascular volume. Fluid lost as sweat, even though hypotonic, may lead to large electrolyte losses in proportion to the magnitude of sweat loss (Briggs, 1975).

The chronic exposure to heat in the tropics, requires a shift of blood flow from the viscera and the body core to the skin and muscles of the extremities, together with an increased sweating to facilitate adequate thermoregulation. This may cause a marked decrease in the volume of the extracellular fluid (ECF) or plasma and may lower arterial blood pressure (ABP). The body attempts to compensate for this by an increased retention of electrolytes and water through neural, humoral and renal mechanisms.

Despite these evolved mechanisms to ensure minimal effects on his internal milieu, the acclimatised individual, may be faced with an extra demand for water and electrolyte which may be aggravated by heavy physical exertion (Akinkugbe, 1974). This would definitely affect the pattern of water and salt intake and voiding. Fluid losses via the respiratory and cutaneous routes, in tropical residents, have been shown to be very high compared to those in temperate residents (Badoe, 1968; Elebute, 1969). Electrolyte losses, on the other hand, have been estimated to be minimal (Tinckler, 1966) inspite of the large fluid losses by these routes.

Since sodium is the main determinant of the ECF volume, its conservation should be a major defense mechanism in the maintenance of an adequate ECF volume, especially, in tropical residents.

1.2 Sodium balance and the Extracellular Fluid volume.

Since the volume of the ECF is determined primarily by the total amount of osmotically active components, it is imperative that these substances are conserved or replenished during periods of appreciable losses. Any changes in the ECF or body fluid volume arising from changes in its composition of osmotically active solutes (electrolytes), would eventually be reflected in the haemodynamics of the organism.

The maintenance of the volume and composition of the ECF is basically achieved by an effective renal mechanism which is aided by a hormonal system: the Renin-Angiotensin System (RAS), and an effective cardiovascular response to offset any resulting fluid and electrolyte imbalances in the body. This is usually augmented by mechanisms that allow for adequate intake of fluid and electrolytes.

The major cation, sodium (Na^+), together with its associated anions (mainly chloride), determine to a very large extent the osmolality and volume of the ECF (Rowell, 1986). The amount of Na^+ in the ECF determines the osmotic gradient for the movement of water in the body. This would mean that, any appreciable changes in the amount of Na^+ in the ECF or plasma would, in the long run, affect ABP by changing the volume of the ECF and hence that of blood in circulation. Since the quantity of Na^+ in the body at any time is a balance between Na^+ input or intake and its output or excretion, it is important that Na^+ intake must match output to forestall changes in body Na^+.

The intake of Na^+, like food, is affected by many factors such as the individual's genetic or organic constitution, as well as psychosocial influences (Hollenberg, 1984). However, due to the ready-availability and cheapness of common salt (NaCl), Na^+ intake in most populations (Westernised) is far in excess of body requirements. Furthermore, the acquisition of "western" dietary habits, coupled with the continuous indulgence in certain cultural dietary practices allowing excessive use of salt, all

contribute to an excess intake in some populations. In such circumstances, intake may exceed output and accumulation of Na^+ is likely to result. This may eventually lead to an increase in body fluid volume and ABP (Young et al., 1977). Thus, dietary salt may be considered as an important determinant of ABP.

In health, Na^+ balance is maintained mainly through its excretion via the kidneys with a variable contribution in sweat (Mclaren, 1985). The excretory function of the kidney and alterations in renin secretion rates via interrelated endocrine functions determine the body sodium content (Laragh & Sealey, 1973). The kidneys therefore play a central role in the regulation of sodium homeostasis and ABP. On the other hand, alterations in sodium homeostasis and ABP have direct effects on the kidney. For instance, an increase in ABP results in an increased urinary output of both fluid (diuresis) and salt (natriuresis), to bring the ABP back to normal levels (Hall et al., 1990).

The quantities of Na^+ lost in sweat are largely dependent on physical activity and ambient temperature. During heavy physical activity or chronic exposure to critical sweating temperatures, sodium loss through the sweat may increase in proportion to the magnitude of sweating (Briggs, 1975). However, when such obligatory losses occur, individuals may show a craving for salt which results in an increased salt intake (Fitzsimons, 1972; 1980). Normally, there are limits to Na^+ excretion and to the ECF volume that can be achieved by altering sodium intake (Hollenberg, 1984). At low levels of sodium intake, urinary sodium excretion approaches zero. On the other hand, at high levels of intake, when the ECF volume is replete, sodium excretion may match intake. Over a wide range of dietary intakes, the renal mechanism is modified by the RAS to achieve additional optimization in the control of the body fluid volume and ABP until secondary effects set in (Hollenberg, 1984).

Salt balance and water balance determinations are associated in the maintenance of body fluid volume and hence the ABP. The control of water intake and output

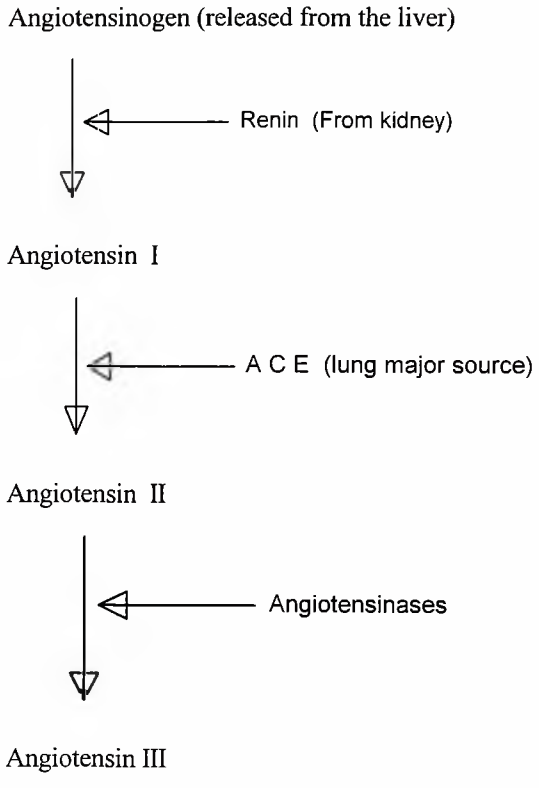
resembles that of sodium in terms of the mechanisms involved and the major stimuli that elicit them. The sensations of thirst and salt appetite on one hand, and the renal excretion of water and electrolytes on the other, are affected by changes in the levels of vasopressin and hormones of the RAS. Since alterations in body fluid volume and the ECF volume would affect ABP, it is most likely that any factors that alter these fluid volumes could modify the ABP (Rowell, 1986).

1.2.1 Sodium, the Renin-Angiotensin System and arterial blood pressure control.

In the past years, it has become apparent that the RAS plays a vital role in the regulation of salt and ABP homeostasis (Laragh et al., 1972). The RAS, through synergistic actions of vasoconstriction by angiotensin II (Ang II), that influences the circulation directly, and indirectly through sodium retention by the action of aldosterone and other hormonal systems, is a major regulator of both sodium homeostasis and blood pressure (Hall, 1986; Hall et al., 1990).

Angiotensin II, an octapeptide resulting from the sequential cleavage of a circulating plasma protein, is one of the most potent natural vasoconstrictors to be identified. It is obtained from the actions of renin, a kidney enzyme, and angiotensin converting enzyme (ACE). The cells of the juxtaglomerular apparatus release renin which is known to act on a circulating alpha globulin, angiotensinogen, converting it to the decapeptide Angiotensin I (Ang. I). Renin is released from the juxtaglomerular cells of the kidneys by stimuli that include a fall in renal perfusion or in sodium supply to the distal tubules and an increased renal sympathetic outflow. The Ang. I formed is then hydrolysed by ACE, an enzyme mostly found in the lungs, to yield the active Ang. II. This sequence of conversions is illustrated below in figure 1.

Figure 1. Schematic presentation of the Renin-Angiotensin System (RAS).



Angiotensin II is known to have both direct and indirect pressor effects. Directly, it may act on both the central and peripheral nervous systems. In the central nervous system, Ang. II acts on the area postrema and the subfornical region of the third ventricle, where it is postulated to raise the ABP through increases in sympathetic outflow (Buckley and Jandhyala, 1977) and vasopressin release (Simpson, 1981) as well. It is also known to elicit a potent thirst response, salt appetite and adreno-corticotropin release (Fitzsimons, 1980) by acting in these areas. These findings suggest that Ang. II is a modulator of water and salt homeostasis, and through these actions affect ABP. Furthermore, the effects of Ang. II on the central nervous system may show what appears to be a localised RAS of the CNS (Brody and Johnson, 1980).

Peripherally, Ang. II is known to elevate ABP by direct vasoconstriction and also by stimulating the zona glomerulosa cells of the adrenal cortex to release aldosterone (Ames et al., 1965). It is also known to modulate the release of neuro-transmitters from sympathetic nerve terminals (Hofbauer et al., 1983). In addition, it has definite effects on afferent arteriolar resistance, glomerular filtration rate (GFR), intrarenal distribution of blood flow; so as to alter the renal excretion of sodium (Hall et al., 1986; 1990).

The hormone aldosterone, mostly released by Ang II, is known to potentiate sodium reabsorption throughout the renal tubules (Hierholzer and Stolte, 1969). It was first identified in 1955, and has been known to have a life sustaining role in the renal regulation of sodium balance (Laragh, 1973). According to Laragh (1973), in 1960 this adrenocortical secretion was revealed to be part of a control system for co-regulating electrolyte balance and ABP. The production of aldosterone is known to be stimulated by several factors including low plasma sodium levels (hyponatraemia), high plasma potassium levels (hyperkalaemia), corticotropin (ACTH) and renin (acting via Angio-tensin II). However, none of these acting individually could account for the observed increases in aldosterone production (Boyd and Peart, 1971). In 1972, Boyd and his colleagues (Boyd et al., 1972) showed that, even though Ang. II was only partially

responsible for the secretion of aldosterone in salt depletion, its role was significant and of particular interest in the overall regulation of the ECF volume.

The secretion of both renin and aldosterone is known to fluctuate in parallel with changes in dietary salt content (Laragh, 1973). Secretions of both renin and aldosterone are also known to reach maximum levels with extreme sodium deprivation and decrease to a minimum with sodium excess.

Laboratory determinations of Plasma Renin Activity (PRA), which is a reflection of the rate of renal secretion of renin, can be used as an indicator of the renin axis's pressor activity supporting ABP, as well as sodium retention through the actions of Ang. II and aldosterone (Laragh et al., 1972). In a recent experiment by Volpe and his associates (Volpe et al., 1993), a high salt diet was found to be associated, not only with a low PRA, but also a low level of aldosterone and an increase in the level of plasma Atrial Natriuretic Peptide.

Studies in black populations have shown the presence of inappropriately low levels of PRA, which may be attributed to a possible impairment of the renin secretory process (Fray et al., 1987). However, low renin secretion has been thought to be a physiological response to an increased plasma sodium concentration as well as volume expansion (Tobian, 1960; Overbeck et al., 1981), which may be linked to an increased salt intake (Sagnella et al., 1990).

1.2.2 Other sodium related factors in the control of arterial blood pressure.

In 1952, before the RAS was recognized, Peters (Peters, 1952) had proposed an osmoregulatory system that was supposed to work by perceiving changes in the blood volume and eliciting appropriate changes in sodium homeostasis. Later on, Homer Smith (1957) in a classic essay, considered the atria as probable sites for such volume regulators. In later years, de Wardener and coworkers (de Wardener et al., 1961) as

well as other researchers went on to investigate and elucidate the effects of a humoral agent other than aldosterone, and to characterise this putative hormone capable of causing natriuresis after saline loading (de Wardener and McGregor, 1980).

However, it was not until 1981 that de Bold and colleagues' (de Bold et al., 1981) landmark experiment led to the location of atrial natriuretic peptide (ANP) in atrial granules. It was demonstrated by these workers that, extracts of atrial tissue when given intravenously to rats, could cause very large sodium diuresis (natriuresis), and lower ABP. Meanwhile, earlier findings had shown that an increase in right atrial pressure stimulated urinary salt and water excretion (Henry et al, 1956) and then, much later, the increase in right atrial pressure stimulated the secretion of atrial natriuretic peptide (Lang et al., 1985). All these findings led to the discovery of ANP.

Atrial natriuretic peptide is a potent vasodilator of pre-constricted blood vessels. As a natriuretic hormone, its effects may be mediated by its renal haemodynamic action by way of an increase in glomerular filtration rate. Atrial natriuretic peptide also has four discrete anti-RAS actions (Laragh, 1969) that include a reduction in renin secretion, a blocking of aldosterone secretion, an opposition to the vasoconstrictor effect of Ang. II and finally opposing the sodium retaining action of aldosterone. All these actions could be of primary importance in the control of long term sodium balance and ABP.

1.2.3 Sodium and hypertension.

There is ample evidence to show the association between excessive sodium (salt) intake and the elevation of arterial blood pressure or hypertension. However, suggestions about the existence of this relationship in various societies were made by a Chinese physician as far back as 2300 BC (Shunyu & Huang, 100 - 200 BC; cited by Swales, 1975).

About two decades ago, evidence was obtained to show that very high NaCl diets raised the ABP in almost any human (Murray et al., 1978). Subsequently, cumulative epidemiological evidence has shown a trend in the prevalence of hypertension in populations noted for a high intake of salt (Gleibermann, 1973; Swales, 1990) and the virtual absence of hypertension in populations with very low salt intake (Page et al, 1976; Akinkugbe, 1972). Presently, the literature is inundated with experimental work on single populations as well as groups of populations, that lends support to this hypothesis (Muntzel & Drueke, 1992; Stamler, 1993; Beard, 1994; MacGregor, 1994). These studies, which were done on either humans or animals, together with data from epidemiological surveys (Swales, 1990; Elliot, 1991), have highlighted the pressor effect of salt loading which was first demonstrated by McQuarrie et al (1936). Much relevance has been given to the role of salt in hypertension, with the realisation that a low salt diet was beneficial in lowering the arterial blood pressure (Beard et al, 1982; Law et al., 1991a; 1991b; Frost et al., 1991).

Considering the mechanism by which sodium raises the ABP, there are as many hypotheses as there are researchers in this area. So far, the possible ways by which sodium may cause ABP elevation include both direct and indirect effects. Whilst some have considered the expansion of the ECF volume resulting in a sustained increase in cardiac output, as the cause of the elevated ABP (Laragh, 1973; Gavras, 1982), others point to a sodium induced alteration of vascular wall structures (Tobian and Binion, 1954). In an experiment by Bohr and Berecek (1976) sodium was shown to cause a rise in ABP by inducing other pressor agents; by causing their secretion or enhancing their activity. Yet another experiment has shown that sodium may have a unique pressor effect, due at least in part, to a neurogenic mechanism mediated through the activation of the central sympathetic nervous system (Gavras, 1986).

Some other putative mechanisms have centred on abnormal cellular transport mechanism involving sodium (Poston, 1987; Houston, 1986; Blaustein & Hamlyn,

1983; Lau et al., 1992). However, according to Guyton and coworkers, (Guyton et al., 1990) sodium may have a direct constrictory effect on the arteries and may, indirectly, alter the secretion of hormones that are responsible for the neural control of circulation as well as cause volume loading by causing secondary fluid retention in the body. In actual fact, fluid retention, regardless of its cause, has been shown to be more important in inducing hypertension through volume expansion (Freis, 1976). However, it is well known that volume expansion of ECF is more often caused by retention of salt (Norman et al, 1975).

Since the kidneys regulate salt and water excretion, in response to a variety of stimuli, any kidney that may have a decreased ability to effectively excrete a salt load, would cause sodium retention with resultant volume expansion and, eventually, a raised ABP. This defect has been found to exist in genetically susceptible groups of animals and humans (Dahl et al, 1967, Tobian, 1990), and is of considerable importance in inducing hypertension. In Ghana, work done by Arthur and colleagues (Arthur et al., 1991) strongly suggests the presence of this group. However, it is believed that the combination of a kidney with a decreased ability to excrete a salt load plus a high salt diet, is what brings about the elevation of ABP.

Considering that the present status of sodium, both as a physiological and environmental stressor, is very much recognised (Simpson, 1990), it would not be out of place to focus attention on mechanisms of sodium homeostasis as determinants of ABP in the tropical resident.

1.3 Determinants of arterial blood pressure.

Several studies have been undertaken in both westernised and traditional non-western populations regarding ABP. Most of them have been concerned with evaluating or assessing casual blood pressures in relation to some other physiologic or pathologic parameter of the population. More often, the assessment of genetic and environmental diversity that characterise the population is omitted. While the effects on BP by such factors as salt (Oliver et al., 1975) or psychosocial stress (Henry, 1988) have been assessed, the focus on other equally important factors like genetic adaptations and peculiar cultural and historic factors, which may significantly impinge on the specific factors like salt and stress, has been minimal (James & Baker, 1990).

The successful adaptation of individuals in a population to specific transient or pervasive environmental stressors is crucial for the survival of the population. Such stressors could affect the population by altering the gene frequency through differential survival of specific genotypes or by modifying the phenotypic response of individuals. The population may also adapt by adopting cultural practices which buffer individuals from the environmental insult (James & Baker, 1990).

According to Sir George Pickering, BP is inherited like a quantitative genetic trait (Pickering, 1961). It could also be regarded as a measurement describing a dynamic state determined by interactions of many traits which function in concert to maintain some central value. As a dynamic phenomenon, it changes from moment to moment as warranted by environmental conditions; with an average value determined by the genetic mechanisms that define these parameters and the stressors that affect their function (Zacharia et al., 1990; Pickering, 1988; Uusitalo et al., 1988).

Like most physiologic variables, ABP has been observed to oscillate within a period and to exhibit rhythmic patterns of varying frequencies (Carendente and Halberg, 1985). The recognition of this rhythmic phenomenon in BP is becoming increasingly

acceptable by most physicians and, very soon, the serial measurement of an individuals BP over a specified time frame, will be much useful in augmenting the diagnosis and treatment of high blood pressure (Pickering, 1988).

Despite the observed temporal oscillations, BP is maintained within physiologic limits by mechanisms which ensure that adequate tissue perfusion is not jeopardised. Furthermore, BP can be thought of as a response to genetic and environmental factors that;

a) is regulated by several endocrine mechanisms,
b) depends upon the extent and stiffness of the arterial tree, and
c) can be modified by other factors that affect body fluid volume (Rowell, 1986).

So that, the individuals susceptibility to any pathological changes arising from the alterations in the ABP would reflect the levels of that individuals BP. These descriptions of ABP reveal it as a phenomenon that can be modulated by factors emanating from both within and without the individual.

With regards to body fluid or ECF volume regulation, the kidney plays a very dominant role. By its excretory function, as well as through other interrelated endocrine functions that alter renin secretion rates, the kidney determines body sodium content and therefore body fluid volume (Laragh and Sealey, 1973; Laragh and Resnick, 1988). In the first instance, the ECF volume is manipulated, which then affects the blood volume, cardiac output and finally blood pressure. In the second, the RAS is activated to bring about an increase in pressure by direct effects of constricting the peripheral arterioles, as a short-term regulatory measure, and by a further manipulation of the ECF volume by indirect effects. It is these two mechanisms that give the kidney its overriding power on other BP control mechanisms (Guyton et al., 1970; Guyton, 1980; 1990) and, generally, allow the expression of its effects in the long term. It is possible that a long period of exposure to the prevailing tropical environment and other stressors, which are

becoming more and more recognised, may alter the genetic factors of acclimatised individuals and subsequently become important determinants of their ABP.

1.4 Background to the study.

In a tropical country such as Ghana, it is well established that prevailing weather conditions could result in losses of large amounts of salt in sweat. Badoe (1968) has evidence from restricted subjects, which shows that losses of fluid alone through the respiratory and cutaneous route under such conditions could be higher than expected. So that, losses from ambulant normal persons would be appreciably higher. Such quantities of fluid lost would be in proportion to sodium losses.

For individuals to maintain reasonable sodium balance and survive under the prevailing tropical conditions, it is imperative that they evolve buffering mechanisms that would adapt them to the constant changes in fluid and electrolyte homeostasis. Various studies have elucidated certain adaptive physiological mechanisms evolved in individuals under hot climates. These adaptations are mostly aimed at minimising the loss of sodium and water as well as conserving them. An increased secretion of vasopressin (Collins, 1963) as well as a possible increase in aldosterone secretion (Streeten, 1966) under hot conditions would help minimise the obligatory losses in sweat. Addae & Addae (1970) have also observed a reduction in the GFR of Ghanaians resident in the tropics, which suggests a strategy to conserve these substances or minimise their obligatory loss through the urine. This calls for a kidney that would virtually reabsorb all its filtered load of salt or excrete this load at a slow rate.

Adding to these physiologic adaptations are those psychosocial strategies, often adopted as cultural practices, that tend to ensure adequacy in the internal environment of those substances that are lost under the tropical conditions. Such practices include the use of salt for preservation, adding salt at cooking and at table, that is, increased salt usage..

In present times, the availability and easy access to salt has enhanced the existing practice of using salt for preservation both in industry and traditionally. Such a

situation could lead to a high salt intake which may result from an acquired increase in the salt taste threshold of, especially, urban and coastal dwellers.

For the adapted resident whose kidneys are apt in conserving salt and water, such an excessive intake may result in a volume expanded state since the kidneys would be slow in ridding the body of the excess load. The effect in altering ABP would be such that susceptible individuals may adapt to the pathological changes emanating from the alterations, and may adapt to ABPs that are elevated.

This may explain the recent observed increases in the prevalence of hypertension in a tropical population group that is predominantly urban-coastal (Ikeme et al., 1978) and may also explain the observed differences in the prevalence levels between urban and rural dwellers in Africa (Muna, 1993). Other factors accounting for this trend include changing lifestyles as well as environmental and socio-economic variables.

In view of the above, the aim of this study is to establish the adaptive value of sodium homeostasis and its regulation by the RAS in tropical residents and to study any possible link between this adaptation and hypertension.

1.5 Hypothesis

The observed increases in the prevalence of hypertension in Ghana, a tropical country, is due to an increased intake of sodium in individuals physiologically adapted to conserving sodium.

CHAPTER TWO

MATERIALS AND METHODS

The experiments were designed to follow three serial protocols to facilitate the use of the same sampled population. Human subjects were used in all the studies. All measurements as well as sample collections were done in subjects' normal residence without restricting their normal routines. This was necessary to ensure minimal physiological alterations during the course of the study. In all series, the week prior to the study was used in briefing and familiarising subjects on the experimental procedures.

2.1 Experimental protocols.

Three experimental protocols were followed and are summarised in Table 1 below.

Table 1. Summary of experimental protocols.

Protocol	Type of Measurement/Study
Series I	Haemodynamic measurements and Analysis.
Series II	Plasma Renin Activity determination and analysis.
Series III	Water and sodium balance determined by intake and output analysis.

The following procedures were used for each serial study.

2.1.1 Series I: Haemodynamic study.

In this series, casual blood pressures (Systolic -SBP, Diastolic - DBP) and pulse (PUL) of subjects were measured for 14 days. The subjects were selected randomly from the Ablekuma subdistrict of Accra. The randomised selection of subjects was facilitated by the use of random sampling numbers obtained from a table (Fisher and Yates, 1972).

This procedure was adopted to ensure that any individual was selected independently, and had the same probability of being selected as any other individual. To this end numerals chosen from the table of random numbers were serialised, and subjects were selected in that order mainly from the adult out-patients of the Mamprobi Polyclinic.

On each day, out of an average of 60 patients attending, only about 10 subjects were selected. Those selected were followed up in their respective homes where the measurements were done. The taking and recording of subjects blood pressures were done at least 2 to 4 weeks after they had recovered from their symptoms and had returned to normal good health.

2.1.1.1 Measurement of Blood Pressure.

Prior to the start of measurements, subjects were allowed about 5 to 10 minutes of rest. Daily blood pressures and heart rates (pulse) of subjects in the sitting posture were measured and recorded for at least 14 days. These measurements were done between 0700 and 0900 hours of each day, using an electronic digital blood pressure monitor (Lumiscope Digitronic, 1060. Japan). The digital blood pressure monitor is designed to measure BP by the oscillometric method with a high precision (only about 2% error of measurement). Duplicate measurements were taken in quick succession.

For all measurements, each subject was seated with the elbow rested on a table so that the upper arm was at the height of the heart. The subject was made to relax and remain still during measurements. An appropriately sized arm cuff was placed around the left upper arm snugly so that the bottom edge of the cuff was positioned one inch above the elbow joint. The cuff was placed so that a finger could be inserted beneath and then it was pressurised with the rubber bulb.

From the SBP and DBP recorded, Mean Arterial Pressures (MAPs) were calculated using the equation below:

$$MAP = DBP + {}^{1}/_{3} \text{ Pulse pressure (PP)}$$

where $PP = SBP - DBP$.

The various blood pressure profiles, including pulse and calculated MAPs for all subjects, were then tabulated (see appendix A) to facilitate statistical analysis.

2.1.2 Series II: Plasma Renin Activity study.

In this series, PRA was determined for 10 subjects on seven successive days, with a day interrupting. The subjects included 4 males and 6 females, 2 of whom were hypertensive. Selection here was based on willingness to participate and all subjects were chosen from the randomly selected participants in series I. Blood samples were collected whilst subjects were in good health. Each subject was briefed thoroughly on the objectives of the study and his or her verbal consent sought prior to the commencement of the study.

2.1.2.1 Collection of blood samples.

Subjects were made to rest for about 10 minutes each day in the sitting posture before venous blood samples were collected. The blood pressure and pulse were measured before and after each blood collection, using the same procedure as in series I. All these were done between 0700 and 0900 hours each day.

From each subject, about 2 ml of blood was collected daily by venipuncturing of antecubital and/or radial veins. This was done using disposable 5 ml syringes and 21G x 1.5 inches needles (GHAMED). The blood samples were then put into vacutainer tubes that had been previously coated with Na-EDTA and placed on ice in an insulated ice container. The Na-EDTA prevented the blood from clotting, whilst the ice lowered the rates of enzymic degradation of angiotensinogen. The blood samples collected were later spun in a refrigerated centrifuge (DENLEY, BR401) at 1000 x g centrifugal force for 10 minutes and at 4 degrees Celsius (^{o}C). The plasma formed was pipetted into 5 ml capped plastic tubes sitting on ice, and were later stored at minus (-) 20 ^{o}C until time of assay.

2.1.2.2 Plasma renin activity determinations.

The method of PRA determination described below is similar to the one used by Mendelssohn (1976). PRA has been defined as the amount of Ang. I generated or formed after incubation of sample (plasma, etc.) at a required temperature for a specified period (Mendelssohn, 1976). Angiotensin I has often been measured in preference to angiotensin II concentrations, because the latter is highly unstable and has a relatively short half-life. The amount of Ang. I generated from the incubated mixture is then determined by Radioimmunoassay (RIA) and the PRA is expressed in the following units: Concentration of Ang. I per millilitre per hour ([Ang. I]/ml/hr).

a) Incubation procedure.

Materials required include:

- Buffer A at pH 6.5 (see appendix B for preparation).
- Angiotensinase/ACE Inhibitors.
  - [2,3 Dimercaprol (1.25 mmol/l).
  - Phenylmethylsulphonyl Fluoride (PMS), (4.6 mmol/l).
- Plasma - 100 μl/tube.

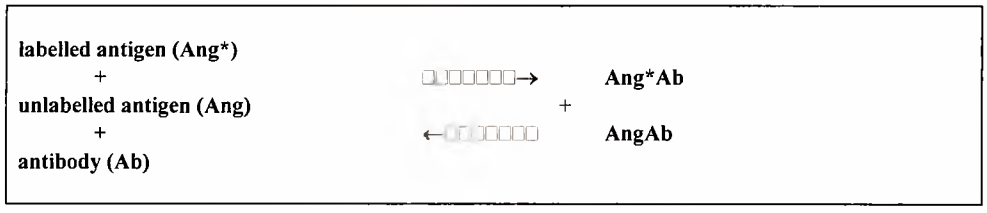
The mixtures, containing 100 μl of plasma and 370 μl of buffer A, were adjusted with equal volumes of angiotensinase and ACE inhibitors to a final volume of 500 μl. The angiotensinase and ACE Inhibitors were added to block the conversion of Ang. I to its metabolites.

The mixture was incubated at 37^{o}C in a water bath (LTE UNITEMP) for 4 hours and then the reaction was stopped by boiling for 30 seconds. The boiling process inactivated the renin enzyme. The mixture was then allowed to cool on standing and 100 μl portions of the supernatant were used for the radioimmunoassay of Ang I. All

pipetting was done using air displacement micropipettes (WHATMANS ABSALES, UK).

b) Determination of plasma levels of angiotensin I.

Measurement of plasma levels of Angiotensin I were done using the RIA technique. The principles of RIA are derived from immunology. It is an analytical method that combines the specificity of an immunoreaction (antigen-antibody complex) with the sensitivity of a radiochemical method. The hormone or ligand of interest acts as an antigen to which a specific antibody has been raised. The classical immunological system originally described in 1964 by Yalow and Berson is based on the competition between unlabelled antigen and the corresponding radio-labelled antigen for a limited number of antibody binding sites. This is briefly illustrated by the angiotensin (Ang) reaction below.



The experimental conditions are chosen such that there is a fixed amount of antibody (Ab) and a relative excess of antigen (Ang & Ang*). When equilibrium is reached, there will remain some free antigen and some bound in a complex with the antibody. If the amount of labelled antigen is fixed, the percentage of this which becomes bound will decrease as the amount of unlabelled antigen in the sample is increased. This is a consequence of the competition between labelled and unlabelled antigens for binding sites on the antibody. To assess unknown amounts of the substance of interest, a standard curve is produced. This is achieved by using a range of known amounts of unlabelled antigen in the reaction and obtaining what is known as a binding-inhibition

curve (Standard curve). Unknown samples will then produce an inhibition of binding of labelled antigen which can be read off the standard curve, thus, giving the amount of substance present.

c) RIA Protocol.

In line with the outlined principles above, the RIA of Ang. I generated from the plasma incubates was performed using the protocol provided by Amersham Inc. UK. Materials included:

- 0.05M Tris/HCl buffer, pH 7.5 [plus 0.3% Bovine Serum Albumin (BSA)] (see appendix B for preparation).
- Angiotensin I acetate Salt; Human sequence. (Sigma Chem. Co. USA).
- Purified Ang. 1 antibody (rabbit). (Amersham Inc. UK).
- Labelled Ang. I - [(3-{^{125}I}Iodotyrosyl) Ang. I (5-L-Isoleucine)] - approx. 25000 - 30000 cpm/50 ml. (Amersham Inc. UK).
- Activated charcoal [untreated powder]. (Sigma Chem. Co. USA).
- Dextran, Industrial grade. (Sigma Chem. Co. USA).
- Incubate supernatant (100 µl).
- Round bottom plastic tubes [64 x 11 mm]. (LIP {equipment services} LTD).

Initially, a stock standard of 1.3 mg/ml was prepared with double distilled water from a sample of lyophilised Angiotensin I acetate salt (Human Sequence). A range of standards was then prepared using the stock standard. To prepare the range, two concentrations [0.2 nM (0.26 ng/ml) and 2 nM (2.6 ng/ml)] were made from the stock solution using assay buffer as diluent. With these two concentrations, a range of standards, from 2 to 200 fmol were prepared in assay buffer (See Table 2). The anti-serum was also diluted with assay buffer to give a working dilution of 1 in 25000. Performance of the assay was done in duplicates for each plasma incubate or standard used, and for each tube, the reaction mixture consisted of 100 µl aliquot of plasma

incubate or standards, 100 µl of Tris/HCl buffer, 100 µl of purified Ang I antiserum (rabbit) and 50 µl of radio-labelled Ang. I also diluted with assay buffer to yield about 25000 to 30000 cpm. A summary of the procedure is provided in Table 2.

Table 2. Schematic presentation of the assay.

	Reference Std/Sample (µl)	Buffer - Tris/HCl (µl)	Antibody (µl)	^{125}I Ang. I (µl)
Total Binding (Bo)		100	100	50
Non Specific Binding (NSB)		200		"
STANDARDS				
S_1(2 fmol)	10 (0.2 mM STD)	90	100	"
S_2(5 fmol)	25 "	75	"	"
S_3(10fmol)	50 "	50	"	"
S_4(20fmol)	100 "		"	"
S_5(50fmol)	25 (2.0mM STD)	75	"	"
S_6(100fmol)	50 "	50	"	"
S_7(200fmol)	100 "	-	"	"
UNKNOWNS				
Sample 1...n	100	-	"	"

Definitions

Total Binding (Bo)
This is the maximum binding possible between the radio- labelled antigen and the antibody, where there is no reference antigen present.

Non-Specific Binding (NSB)
This is an estimate of the amount of binding which may occur between the radio-labelled antigen and components of the mixture other than with the antibody; it accounts for the background radiation which may occur during counting as well.

The test tubes were covered with cling film or parafilm and were then vortexed using a spinmix vortex (GALLENKAMP, UK) for about 10 seconds. The reaction mixture was incubated at 4°C for 24 hours to allow equilibrium conditions to be established. Later, a millilitre of dextran coated charcoal, which was continuously stirred by a magnetic stirrer, was added. The dextran coated charcoal, which is a separating agent, was prepared by suspending 2.5 g of activated charcoal and 0.05 g of dextran in 250 ml of assay buffer whilst stirring at 4°C for 20 minutes. After adding the charcoal, the mixture was again vortexed and centrifuged immediately at 2000 x g and 4°C for 10 minutes, in the refrigerated centrifuge. Then the supernatant, representing the bound fraction, and the pellets; the free fraction, were separated and both counted using a mini gamma counter (NUCLEAR ENTERPRISES, NE 1600).

From the counts obtained, the percentage bound (%B) was calculated for each tube using the following expression:

$$\textbf{PERCENT BOUND (\%B)} = \frac{\textbf{Total count - count free}}{\textbf{Total count}} \times 100$$

The counts were analysed and a standard curve generated using a computer program by courtesy of the IAEA. The concentrations of Ang. I in the unknown samples were then extrapolated from the curve.

2.1.3 Series III: Balance study.

In this series, water and electrolyte balance studies were done on 10 male volunteers in normal good health. Prior to the commencement of the study, each subject was briefed on the experimental procedure. The subjects were weighed clad in minimal clothing and their heights were measured. Body mass indices (BMI) were determined from these measurements by applying the formula: BMI = Weight in kg/(Height in m)^2.

For 10 days of the study, each subject was allowed food and drink *ad libitum*. Between 0700 and 0900 hours each day, blood pressure was measured and recorded as in series I. Meals were served based on a menu prepare together with the subjects. It consisted mainly of local dishes prepared from a variety of staples. Even though subjects were served three times a day, they were allowed food in between meals, as long as weights of the food and samples were made available for analysis. There was no restriction on salt usage at table as well as on other daily routines including physical activity.

The caloric value of the food eaten was not determined. However, each component of a meal was quantitatively determined (see below for details) prior to service, and about 10 g portions stored in mini polythene bags at -20^{o}C for subsequent fluid and electrolyte analysis. The volumes of water and any other liquids ingested daily, were also measured. Twenty-four (24) hour urine and stool samples were collected concurrently over the period. These were also measured and about 10 g portions stored at -20 ^{o}C for subsequent chemical analysis (The details of sample collections and measurements are shown below).

2.1.3.1 Quantitative determinations.

a) Food and stool.

Both food and stool samples were weighed using a calibrated kitchen scale (Soenhle, UK) measuring to the nearest 5g. During service, the food items were weighed

cumulatively on plates whilst stools voided during the day were collected and weighed in plastic chamber pots lined with polythene sheets. In both instances the receptacles were first weighed empty, and then together with the food or stool. The difference between the initial and final weights was taken as the weight of the sample.

b) Drinks and urine.

Volumes of ingested water, drinks and urine were determined with a calibrated measuring cylinder. However, to facilitate ease of determination, fluids were ingested in cups whose volumes were pre-determined with the measuring cylinder, and the total volume ingested for each day and by each subject was calculated from the number of cups of fluid as drunk by the subjects.

The water content of the food was estimated from the differences in weight between the wet food and the food residue after drying in an oven (BAIRD and TATLOCK LTD, UK) at 80^{o}C for 12 hours or more (i.e. until weight remained constant).

For urine, 24 hr samples were initially collected into a 4 litre plastic container which had been previously cleaned and rinsed several times with distilled water. The volume voided over this period was later determined with the measuring cylinder. This procedure was followed for all the 10 days.

2.1.3.2 Processing of Food and stool samples.

Food and stool samples were processed prior to the determination of electrolyte. The method of processing, known as the wet digestion (ashing) method, was similar to one described in the Analytical Methods of Analysis AOAC (Horwitz, 1975). The reaction required the use of the following reagents;

1. Nitric Acid (HNO_3) - concentrated.
2. Perchloric acid ($HClO_4$) - 70% w/v.

The food or stool sample was finely ground in a clean mortar, after drying at 80°C for between 12 and 18 hours in the oven. An amount of 1g (± 0.05 g) of the dried and ground sample was then weighed into a 100 ml beaker. To the beaker, 5 ml of HNO_3 and 2 ml $HClO_4$ were added respectively. The beaker was then covered with a watch glass, and the solution digested with heating over a hot plate until an almost clear volume of 3 to 5 ml remained. About 10 to 15 ml of distilled water was then added to the digested material and filtered through an acid-washed filter paper into a 50 ml volumetric flask. The filter paper was rinsed with distilled water and the filtrate diluted to the 50 ml volume mark. About 10 ml portions of the filtrate were stored at -20°C for subsequent electrolyte analysis.

2.1.3.3 Electrolyte measurements in food, stool and urine.

Sodium was the main electrolyte determined, and the measurements were done on the pre-digested residues of stool and food samples. However, for the urine samples a direct measurement was done without any further treatment. Analysis of the electrolyte required the use of the Atomic Adsorption Spectrophotometer (AAS). The AAS (PERKIN ELMER, 2280 USA), belonging to the Ghana Standards Board (GSB), was used for these measurements.

The equipment was fitted with a sodium lamp and sodium filter to detect and measure emission of sodium during combustion of samples and standards. Calibration involved the use of standardised solutions prepared in graded concentrations (mmol/l) from purified sodium chloride and distilled-deionised water, which were then aspirated and combusted so that a calibration curve was obtained. During combustion, the intensity of the emission passing through the filter corresponded to the amount of sodium in the sample. Subsequent sample combustions yielded intensities that were extrapolated from the calibration curve, and were recorded as corresponding to the concentration of sodium in the sample. Samples for which emissions fell beyond the detectable limits of

the standard curve were diluted 1 in 10 and the sodium content redetermined as before.

From the values of concentration obtained, for each gram of dried food and stool sample digested, and for each ml of urine sample, the total concentrations (in mmol/l) in each subject's measured sample was determined. Amounts of sodium in mmol were then extrapolated for each subject's sample per day. The values, for water and sodium intakes and outputs for each subject over the period of the study, are shown as tables in appendix C.

2.2 Statistical analysis of data.

For series I, the first and second blood pressure readings for each subject were compared by Student "t" test. Since no significant difference were found, their means were used as the blood pressure for each subject for each day. The overall means and standard errors (SEM) of SBP, DBP, MAP and PUL were computed for each subject, and SBP and DBP were regressed with ages of the subjects. Blood pressure statistics for normotensive and hypertensive groups were compared using a "t" test.

In series II, a two-way analysis of variance (ANOVA) was done to investigate any significant effect of time on Plasma Renin Activity as well as any significant between-subject differences. The PRA values for each subject were also regressed on the corresponding subject systolic and diastolic blood pressure values.

In series III, the mean values for daily water intake and output, for each subject as well as that for sodium intake and output were evaluated as baseline data. One-way ANOVA was used to find any significant differences between subjects for both intake and output variables. Intake and output variables were also correlated with the BMI's and BP variables of the subjects. Correlation statistics were also determined between data extracted for the mean ambient weather conditions prevailing at the time of the

study and the mean daily intake and output variables.

For all statistical determinations, significance was considered below the 5 percent level of probability ($P < 0.05$).

CHAPTER THREE

RESULTS

Altogether, forty-one (41) subjects participated in the studies. The ages of subjects for all the studies, ranged from 19 to 75 years and comprised 23 females and 18 males.

Of the 45 subjects selected randomly for the first study (Series I), only 31 participated. The remaining 14 declined for various reasons. The subjects for the first and second studies were selected from a population of outpatients attending the Mamprobi polyclinic, which is the focal point for health service provision in the South Ablekuma subdistrict of Accra. Ten other volunteer subjects were recruited from the same subdistrict for the third study (Series III), as a result of the declination of the randomly selected group to participate. The results obtained for each serial study are presented below.

3.1 Series I: Haemodynamic study.

3.1.1 Subject characteristics.

The ages of subjects in this series ranged from 19 to 75 years (mean = 39.16 ± 2.75 yr.). These are shown, together with the means for SBP, MAP, DBP and PUL measured for each subject over the period of the study, in Table 3.

3.1.2 Blood pressure analysis.

Out of the thirty-one (31) participating subjects, twenty-one were found to be normotensive and ten hypertensive. The criteria for this classification was based on the WHO definition (1986 WHO/ISH Guidelines for treating mild hypertension, 1986). During the study, all the hypertensive subjects were allowed their medication. Nevertheless, their respective mean SBP, DBP and MAP were significantly higher ($P<0.001$) than those for the normotensives. Furthermore, hypertensives were

significantly older than normotensives (mean ages = 52.7 ± 4.3 cf. 32.7 ± 2.5; P<0.001)). In contrast, the pulse recordings for the two groups were not significantly different. Statistical comparison of variables for the two groups are shown in Table 4.

Regression analysis, using the means of SBP and DBP for each subject against age, yielded significant positive correlation's (For SBP; r=0.661, P<0.001 and DBP; r=0.536, P=0.001). The plots for the regression analyses are shown in figures 2a (SBP) and 2b (DBP) respectively.

The daily blood pressures measured over the period showed significant (p=0.03; ANOVA) variability with time for the sampled population and in both groups. This is illustrated by the plots for the sampled population (Fig. 3a) and the two groups i.e. normotensive and hypertensive (Figs. 3b & 3c respectively). Two other respective plots for a normotensive (Fig. 3d) and a hypertensive (Fig. 3e) demonstrate the occurrence of false positive and false negative blood pressure determinations. Even though the plots depict a daily variability in both SBP and DBP, the measured values tended to decline with time.

Table 3. Subject Ages and means of SBP, MAP, DBP and PUL in series I.

ID	AGE (Yr.)	MSBP (mm Hg)	MMAP (mm Hg)	MDBP (mm Hg)	MPUL (Beat/min)
PAN	24	100.9	75.4	61.8	71.0
GRK	50	104.5	79.9	67.7	65.1
JOB	23	107.0	83.0	70.3	82.3
ABK	24	97.5	79.3	70.4	85.5
ADQ	28	112.2	84.2	71.2	79.5
VIQ	19	118.9	90.2	72.6	80.5
EPA	27	109.5	84.9	72.7	60.4
JAB	24	119.3	89.0	74.0	59.1
BEA	26	119.1	89.1	74.0	72.1
JON	25	120.7	90.2	75.0	58.0
MAT	50	127.0	92.9	76.1	67.3
COF	41	132.6	95.4	76.9	63.2
JEB	50	129.4	94.8	77.7	80.8
ELS	23	115.3	90.5	78.2	83.2
LAB	28	118.6	91.8	79.1	83.5
ABA	45	125.2	94.7	80.0	77.1
WON	26	116.2	92.1	80.1	52.8
ODL	56	119.9	93.4	80.1	75.1
EDT	42	129.6	92.3	82.6	62.4
ELF	35	123.0	98.4	85.8	70.5
PEH	21	129.8	103.8	90.9	86.5
*HYPERTENSIVES					
AUD	67	140.3	102.0	87.9	65.2
CON	75	155.3	110.7	88.5	67.7
SAO	62	165.5	114.1	88.5	80.5
AGB	59	149.4	109.5	89.5	62.3
ELG	44	144.3	110.2	93.1	65.7
EUA	36	155.1	116.1	96.6	73.0
EMM	54	147.7	114.0	97.3	54.0
MEN	43	142.0	114.3	99.8	78.3
SAQ	34	148.0	117.7	102.5	63.9
EMA	53	157.5	121.1	102.8	59.5
Mean SE	39.16 2.75	128.43 3.22	97.26 2.27	82.05 1.93	70.40 1.78

*** Definition is in accordance with WHO criteria (see reference in text).**

Table 4. Comparison of variables measured for the two groups in Series I. Except N and P values, all others are means (± SE).

PARAMETER	BLOOD PRESSURE STATUS		P VALUE
	NORMOTENSIVE	HYPERTENSIVE	
AGE (years)	32.7 (2.5)	52.7 (4.3)	< 0.001
SBP (mm Hg)	117.9 (2.1)	150.5 (2.5)	< 0.001
MAP (mm Hg)	89.8 (1.5)	113.4 (1.7)	< 0.002
DBP (mm Hg)	76.1 (1.4)	95.8 (0.8)	< 0.001
PUL (Bt/min)	72.4 (2.1)	67.0 (2.6)	= 0.542
N	21	10	

Figure 2(a). Plot showing the regression of mean SBP against age in series I

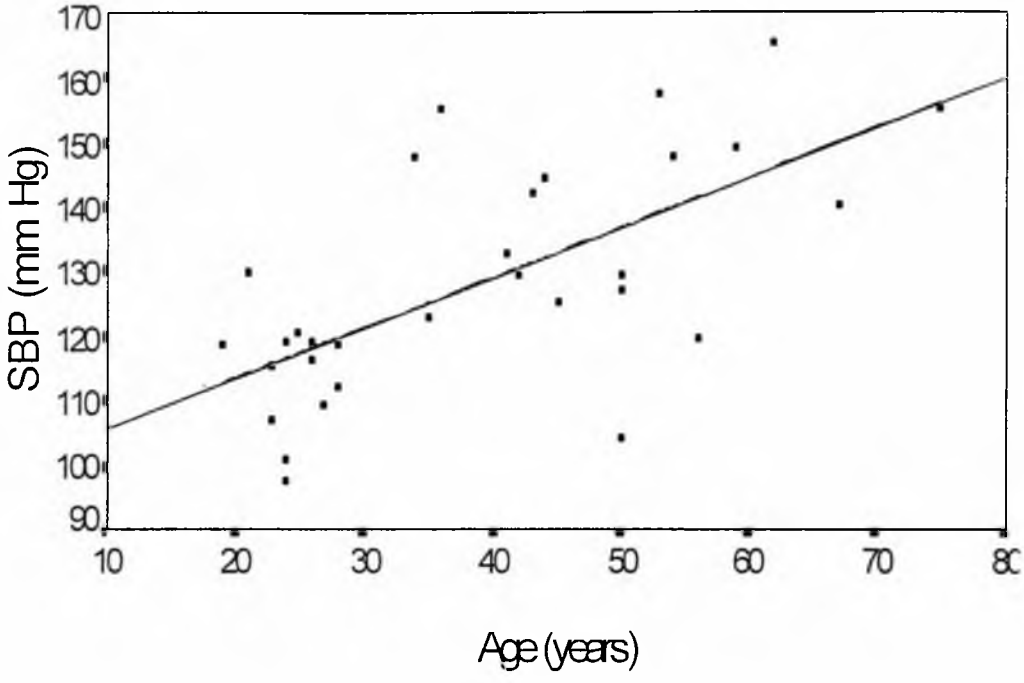


Figure 2(b). Plot showing the regression of mean DBP against age in series I

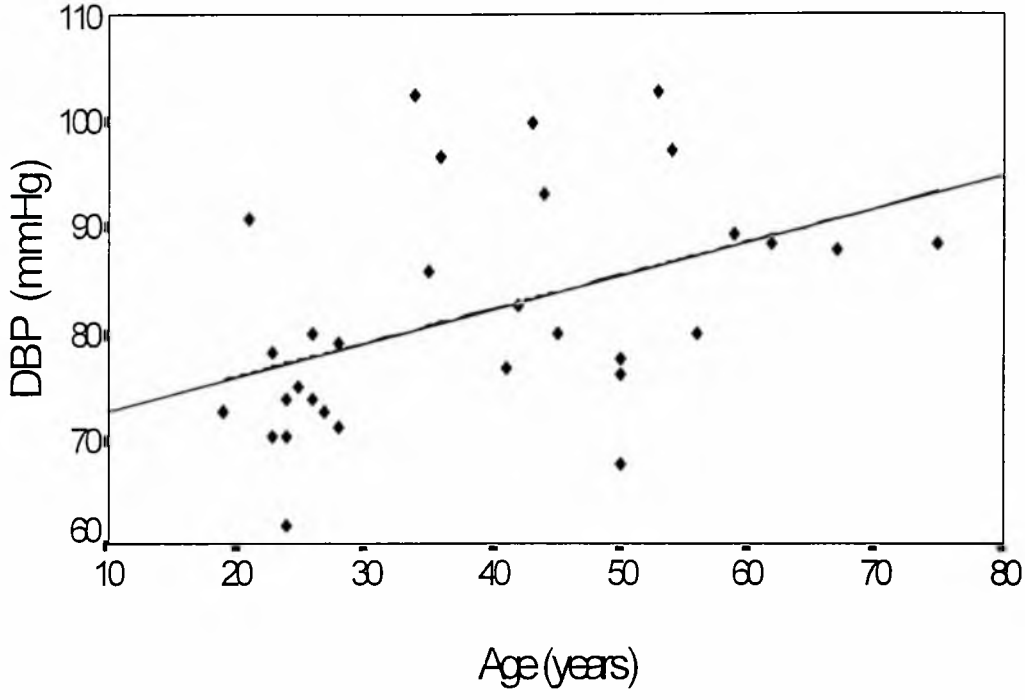


Fig. 3(a). Plot showing the variability of BP in normotensive subjects

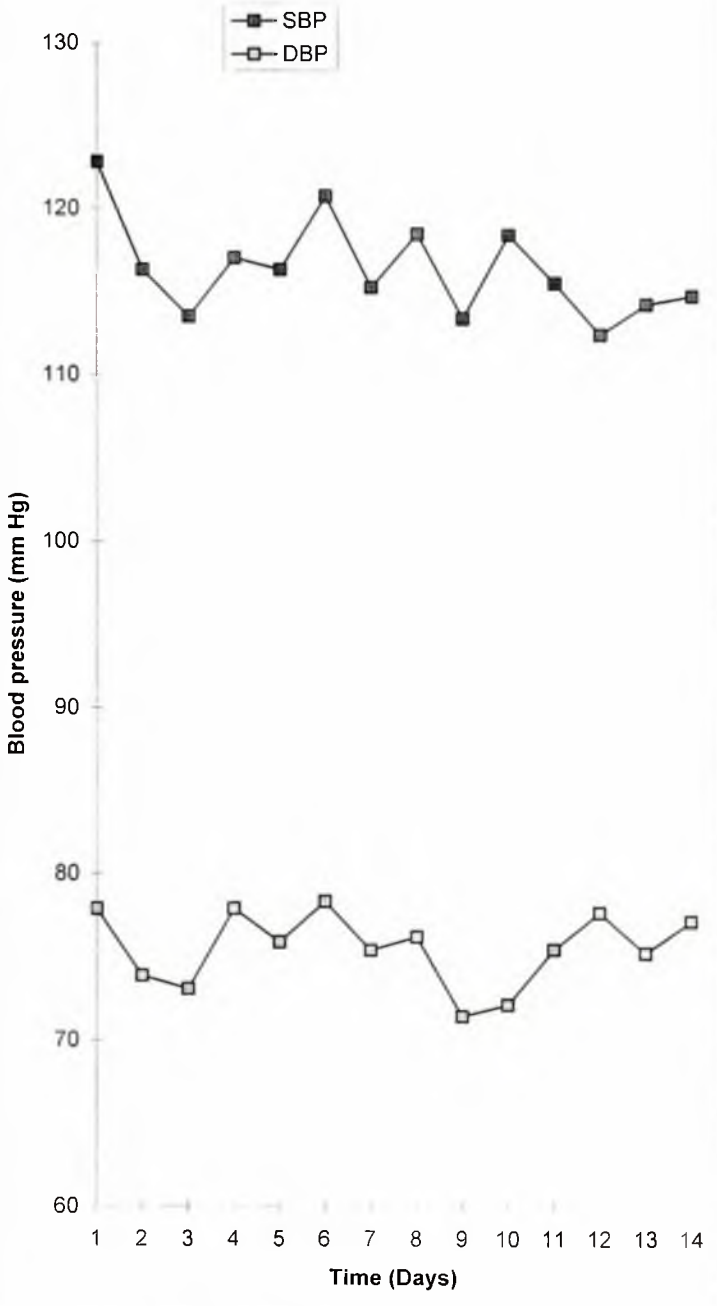


Fig. 3(b). Plot showing the variability of BP in hypertensive subjects

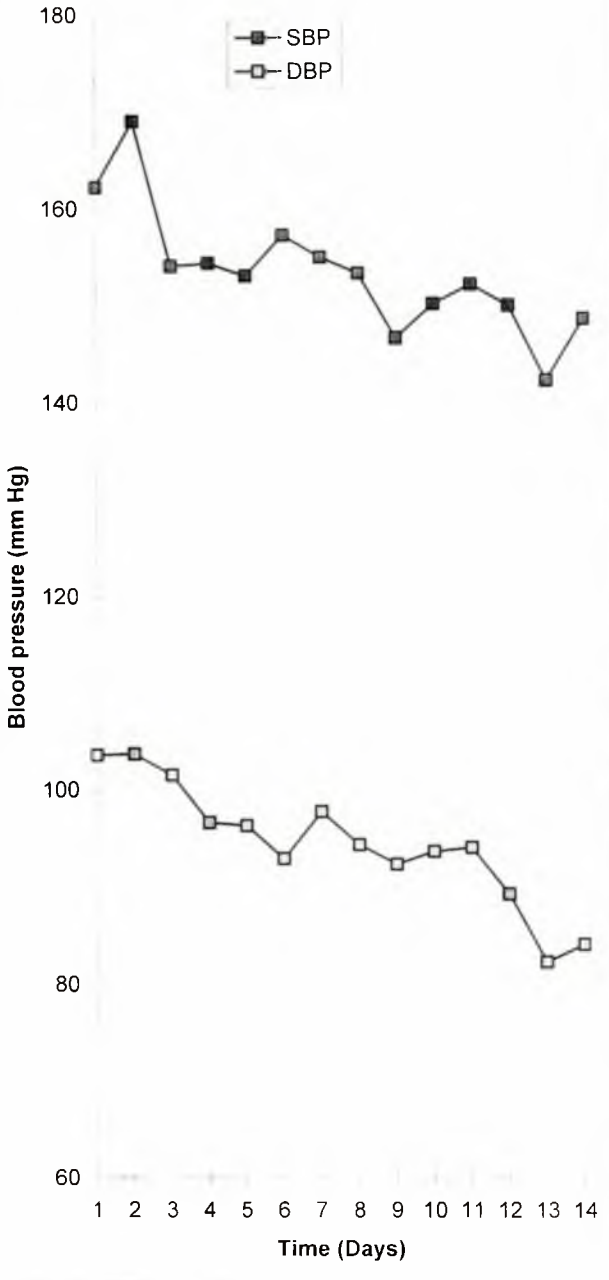


Fig. 3(c). Plot showing the variabilty of BP with time in sampled population

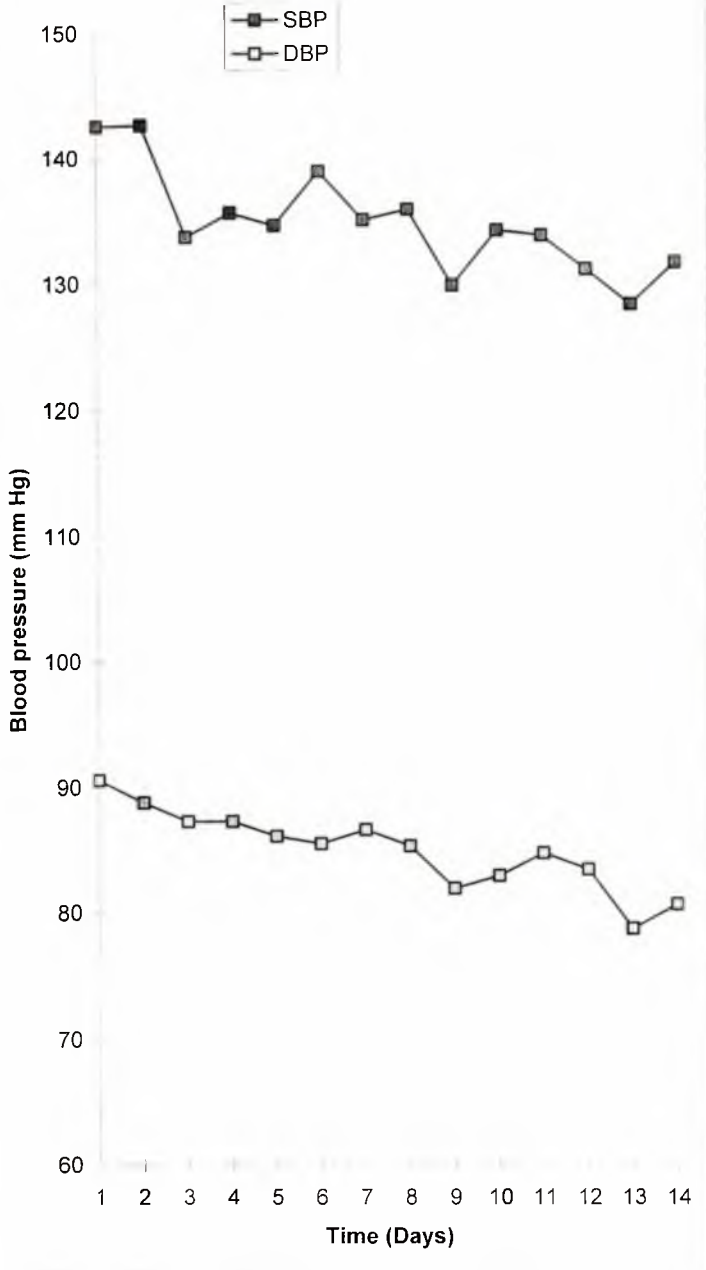


Figure 3(d). Plot showing the varaibility of BP with time in hypertensive subject (AUD)

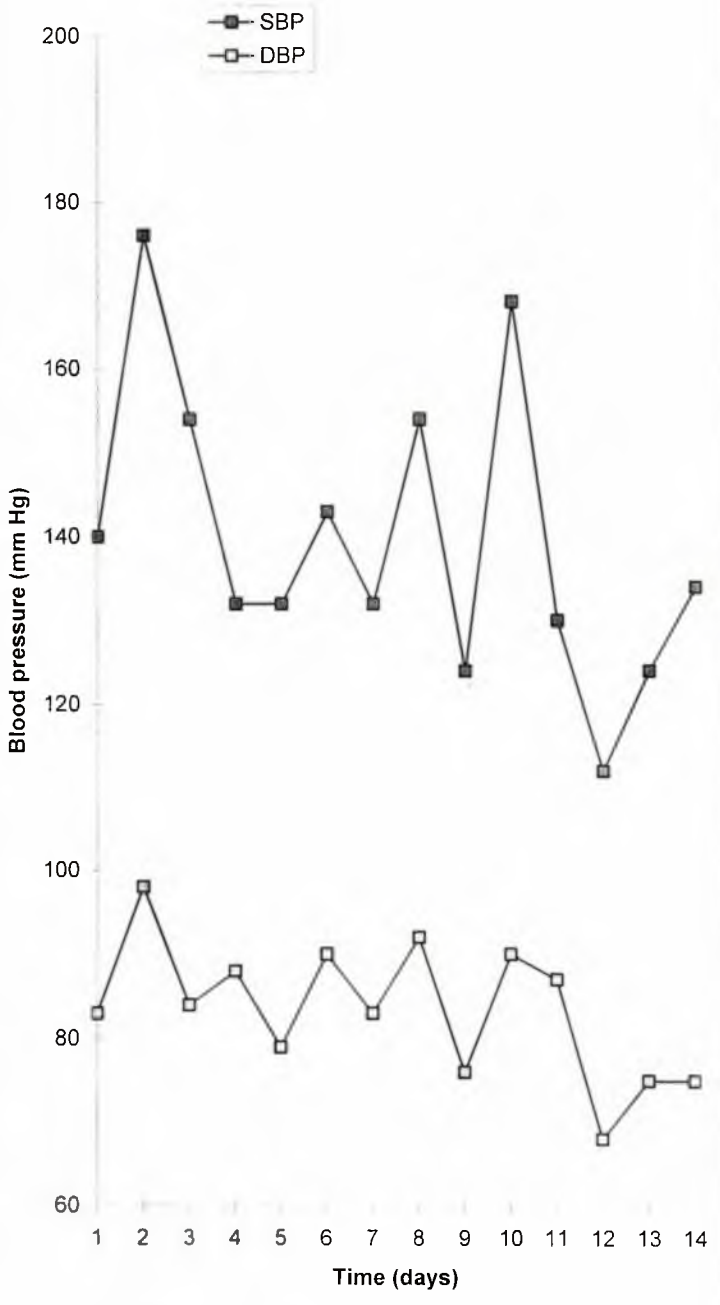
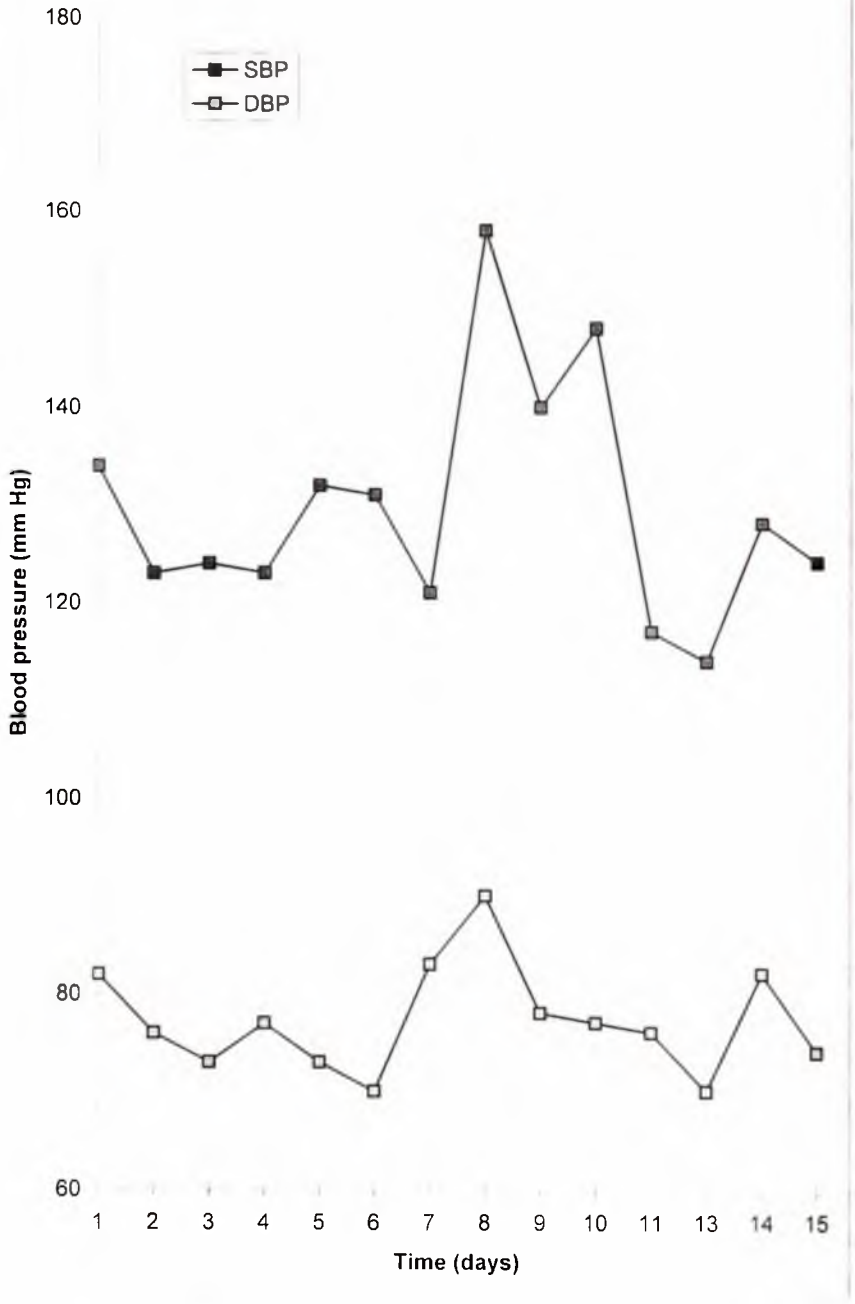


Figure 3(e). Plot showing the variability of BP with time in normotensive subject (JEB)



3.2 Series II: Plasma Renin Activity study.

3.2.1 Subject characteristics.

The 10 subjects studied were aged 21 to 53 years (mean = 33.7 ± 3.8 yr.). Eight were normotensive, comprising 4 males and 4 females, and 2 hypertensive females

3.2.2 Plasma Renin Activity analysis.

Table 5 shows the daily PRA, for each subject for seven consecutive days. A two way analysis of variance showed significant ($P<0.01$) between subject effect, but rather borderline significance ($P=0.057$) for the effect of time.

On the whole, values for each subject's average PRA per day varied from 0.32 to 1.20 pmol; Ang. I/ml/hr (mean = 0.71 ± 0.13 pmol), whereas the average daily range per subject was from 0.62 to 0.86 pmol. A summary of the average subject PRA, corresponding average SBP, DBP and PUL variables, together with the age of subject in the study are presented in table 6. There were no significant correlation's between the PRA values and average SBP, DBP and PUL values. Furthermore, the PRA values between the two groups were not significantly different, even though, the mean SBP and DBP of the hypertensives were significantly higher ($P<0.001$) than that of the normotensives. Unlike the blood pressure variables, the PRA did not show any declining trend with time.

Table 5. **Daily PRA (pmol Ang I/ml/hr) for subjects in series II. * IDs are hypertensive.**

ID	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
*EMA	0.53	0.20	0.45	0.23	0.45	0.42	1.79
*SAQ	0.89	0.21	0.88	0.16	0.27	0.39	0.21
JAB	0.65	0.10	0.78	0.18	0.56	0.51	0.52
ELF	0.47	0.19	0.60	0.18	0.32	0.16	0.42
JEB	0.84	1.33	0.10	1.05	1.27	1.82	1.68
PEH	0.17	1.40	0.64	0.53	0.50	1.48	0.72
ELS	0.93	0.63	0.66	0.50	1.60	2.60	1.48
ABA	0.32	0.56	1.44	0.28	0.13	0.34	0.58
PAN	1.23	0.84	0.77	0.11	0.86	0.33	0.11
EPA	1.22	0.77	0.79	1.70	1.50	0.55	0.38
Mean	0.73	0.62	0.71	0.49	0.75	0.86	0.79
SEM	0.11	0.15	0.11	0.16	0.17	0.26	0.20

Table 6. Age and means for PRA, SBP, DBP and PUL values obtained for subjects in series II.

ID	AGE (yr.)	PRA (pmol)	MSBP (mm Hg)	MDBP (mm Hg)	MPUL (Bt/min)
EMA	53	0.58	152.0	102.0	58.0
SAQ	34	0.43	151.0	104.0	62.0
JAB	24	0.47	123.0	78.0	54.0
ELF	36	0.32	126.0	86.0	72.0
JEB	50	1.15	132.0	78.0	77.0
PEH	21	0.78	132.0	93.0	88.0
ELS	22	1.20	110.0	75.0	83.0
ABA	45	0.52	124.0	83.0	89.0
PAN	24	0.61	103.0	70.0	75.0
EPA	28	0.99	111.0	74.0	61.0
Mean	33.7	0.71	126.4	84.3	71.9
SEM	3.8	0.10	5.2	3.7	4.0

Statistical analysis.

1. Comparison of PRA between hypertensives (n = 2) and normotensives (n = 8) using students ` t' test: T = 1.0207, P = 0.337 (Not significant)

2. **Correlation analysis:**

	r	T	P value
PRA vrs SBP:	-0.350	1.06	0.322
PRA vrs DBP:	-0.451	1.43	0.191
PRA vrs PUL:	0.312	0.93	0.380

3.3 Series III: Balance study.

3.3.1 Subject characteristics.

In this series, there were 10 male subjects who voluntarily opted for the study. Their ages ranged from 23 to 33 years (mean = 28.1 ± 2.2 yr.) with body mass indices (BMI) ranging from 14.5 to 27.1 (mean = 22.4 ± 1.0). Using the WHO criteria, all the subjects in this series were normotensive. The anthropometric parameters are shown together with the blood pressure variables of each subject in table 7.

3.3.2 Balance analysis.

Tables 8 and 9 show the averages for each subject's sodium and water intake, output and net balance. The intake and output averages were derived from daily estimates recorded for each subject over the study period (see appendix C). Values for the net balance were, however, derived from the difference between intake and output. This may represent either losses from the respiratory and cutaneous routes or gains in body sodium and water.

In table 10, the mean input, output and net balance for each day, considering all the subjects together, are shown for both sodium and water, together with the mean diurnal temperatures and relative humidities recorded over the period of the study.

Table 7. **Age, Body Mass Index (BMI) and mean BP variables of subjects in series III.**

ID	AGE (yr.)	BMI (kg/m^2)	BLOOD PRESSURE SBP (mm Hg)	DBP (mm Hg)	PUL (Beat/min)
AME	27	16.7	102	71	84
SBS	23	18.7	127	79	58
NAA	32	23.5	129	88	69
MNC	33	24.8	136	84	57
SLL	27	19.6	131	83	75
WON	26	24.2	133	84	79
MAG	29	20.6	104	73	102
PEN	29	24.2	125	82	59
JAB	25	14.5	127	84	46
PAA	30	27.1	130	83	54
Mean	28.1	22.1	124.4	81.1	68.3
SEM	1.0	1.3	3.7	1.7	5.3

Table 8. Mean sodium input, output and net balance for each subject. Values are in mmol.

	INPUT	OUTPUT			NET
ID	Food	Urine	Stool	Total	BALANCE
AME	267.5	187.9	25.5	213.4	54.1
SBS	319.6	215.8	32.6	248.4	71.2
NAA	314.8	279.8	17.7	297.5	17.3
MNC	321.8	232.0	27.2	259.2	62.6
SLL	307.1	214.4	12.1	226.5	80.6
WON	304.8	213.8	8.8	222.6	82.3
MAG	296.3	217.6	9.4	227.0	69.3
PEN	319.0	235.3	11.2	246.5	72.5
JAB	245.8	200.0	8.7	208.7	37.1
PAA	255.7	212.7	9.0	221.7	34.0
Mean	295.2	220.9	16.2	237.2	58.1
SEM	9.0	9.8	2.9	8.4	6.9

Table 9. Mean water input, output and net balance for each subject. Values are in ml.

	INPUT			OUTPUT			NET
ID	Food	Drink	Total	Urine	Stool	Total	BALANCE
AME	842.0	983.5	1825.5	1138.0	113.9	1252.0	573.5
SBS	1033.0	1708.5	2741.5	1398.0	162.0	1560.0	1181.5
NAA	1030.0	3349.5	4379.5	2376.0	129.0	1480.8	1875.5
MNC	1057.0	1739.0	2796.0	1312.0	168.8	2505.0	1315.2
SLL	985.0	1584.0	2569.0	1507.0	105.6	1612.6	956.4
WON	973.0	1806.5	2779.5	1370.0	85.0	1455.0	1324.5
MAG	932.0	1626.5	2558.5	1430.0	107.0	1537.0	1021.5
PEN	1035.0	2177.0	3212.0	1354.0	131.0	1485.0	1727.0
JAB	1065.0	1674.0	2739.0	1049.0	105.0	1153.0	1586.0
PAA	1068.0	1939.5	2987.0	1385.0	97.5	1472.5	1514.5
Mean	1000.0	1858.5	2858.8	1431.9	120.3	1551.3	1307.6
SEM	22.0	191.5	203.7	113.4	8.6	114.8	123.8

Table 10. **Daily mean diurnal temperature (MDT) and relative humidity (MDrH) recorded over the study period and the water and sodium balance statistics per day for series III. CUM. NET - cumulative net.**

			WATER BALANCE (ml)			SODIUM BALANCE (mmol)			
	MDT	MDrH	INPUT	OUTPUT	NET	INPUT	OUTPUT	NET	
DAY	(°C)	(%)	TOTAL	URINE	BALANCE	TOTAL	URINE	BALANCE	CUM. NET
1	27.3	81.0	3062.5	1076.0	1937.9	211.2	165.1	31.9	31.9
2	27.6	81.5	2500.0	1745.0	628.3	200.9	261.7	-67.9	-36
3	28.7	86.0	3004.0	1342.0	1514.2	268.4	222.9	37.8	1.8
4	28.1	79.0	2787.5	1509.0	1112.9	204.3	196.8	0.4	2.2
5	28.5	85.0	2734.0	1397.0	1228.2	230.6	179.9	33.7	35.9
6	28.3	87.0	3169.0	1273.0	1760.0	347.6	225.0	90.0	125.9
7	28.2	82.5	2943.5	1314.0	1460.5	396.2	163.8	195.5	321.4
8	26.3	87.5	2818.0	1436.0	1270.9	228.0	228.0	-11.1	310.3
9	27.8	85.5	2962.0	1549.0	1298.0	597.5	303.8	274.3	584.6
10	27.8	80.0	2607.5	1678.0	799.5	267.7	262.4	-3.8	580.8

3.3.2.1 Sodium balance.

Prior to the derivation of the averages, a two-way analysis of variance showed significant ($P<0.001$) daily as well as between subject differences for sodium input and output. The means for intake and output are summarised in table 11 below.

On the average, each subject ingested 295.2 ± 9.0 mmol of sodium per day, which was mainly from food. The between subject range was from 245.8 to 321.8 mmol, whereas the daily range was 204.3 to 597.5 mmol.

Urine sodium per subject per day was 220.9 ± 9.8 mmol (range; 187.9 to 279.8 mmol) and from stool, was 16.2 ± 2.9 (range; 8.7 to 32.6 mmol). Daily ranges for sodium output were 163.0 to 303.8 mmol for urine, and 7.1 to 37.9 mmol for stool. An average value for net sodium balance was 58.1 ± 6.9 mmol, with a range of 17.3 to 82.3 mmol per subject and -67.9 to 274.3 mmol per day. Over the entire period, the average net sodium balance, in cumulative terms, was estimated to be 580.8 mmol per subject.

3.3.2.2 Water balance.

Averagely, each subject drank 1858.5 ± 191.5 ml (range: 983.5 to 3349.5 ml) of water per day, while the amount in food was 1000.0 ± 22.0 ml (range; 842.0 to 1065.0 ml). The total average intake, without including endogenous production from the metabolism of ingested food, was 2858.5 ± 203.7 ml with a range of 1825.5 to 4379.5 ml.

Considering the daily variations, water intake as drink and in food per subject ranged from 1586.5 to 2142.5 ml and 607.0 to 1302.0 ml respectively. Daily total intake, on the other hand, varied from 2500.0 to 3169.0 ml. With regards to water output, amounts in urine and stool per subject averaged 1431.9 ± 113.4 ml (range; 1049.0 to 2376.0 ml) and 120.3 ± 8.6 ml (range; 85.0 to 168.0 ml) respectively. The daily

averages, however, ranged from 1076.0 to 1745.0 ml for urine and 48.6 to 168.9 ml for stool. Net water balance was 1307.4 ± 126.2 ml, ranging from 573.5 to 1875.5 ml for each subject and 628.3 to 1937.9 ml for each day. A summary of the outcomes, for which a conservative amount of 400 ml is taken as the water produced from the metabolism of food, is presented in table 12.

Table 11. Summary of intake and output means (per subject and per day) for sodium. Values in mmol. Range per day in parentheses.

Variable	Range	Mean	SEM
Intake:			
food	245.8-321.8 (204.3-597.5)	295.2	9.0
TOTAL		**295.2**	
Output:			
Urine	187.9-279.8 (163.0-303.8)	220.9	9.8
stool	8.7-32.6 (7.1-37.9)	16.2	2.9
Net balance	17.3-82.3 (67.9-274.3)	58.1	6.9
TOTAL		**295.2**	

Table 12. Summary of intake and output means (per subject and per day) for water. Values in ml. Range per day in parentheses.

Variable	Range	Mean	SEM
Intake:			
by drink	983.5-3349.5 (1586.5-2142.5)	1858.5	191.5
by food	842.0-1065.0 (607.0-1302.0)	1000.0	22.0
*metabolism		400.0	
TOTAL	2225.5-4779.5 (2900.0-3569.0)	**3258.5**	
Output:			
Urine	1049.0-2376.0 (1076.0-1745.0)	1431.9	113.4
stool	85.0-68.0 (48.6-68.9)	120.3	8.6
Net balance	973.5-2275.5 (1068.3-2337.9)	1707.4	126.2
TOTAL		**3258.5**	

*** Approximate quantity from endogenous metabolism of food.**

Correlation's of the BMI and ages as well as mean SBP and DBP of subjects, against water and sodium intakes and outputs are summarised in tables 13 and 14 respectively. In table 15, the relation between the prevailing weather conditions and subjects' sodium and water balance are shown as correlations between these parameters and sodium and water intakes and outputs, whilst table 14 depicts correlation statistics between water and sodium ingested against their output via urine and net balance. In addition to the significance shown for DBP against total water intake ($P=0.014$) and net water balance ($P=0.006$), there were also some significant correlations between water drunk and water and sodium outputs in urine as well as between sodium intake and its output via urine (see Table 16). Apart from these, the rest were not significant. For sodium intake and water intake the high correlation ($P<0.001$) obtained was very obvious.

Table 13. Correlation statistics of BMI and age with water and sodium intakes and outputs.

Variables	r	P value
BMI vs.:		
Water intake by drinking	0.4755	0.165
Total water Intake	0.4830	0.157
Urine water output	0.3780	0.281
Stool water output	0.0451	0.902
Net water balance	0.4491	0.193
Sodium intake	0.3734	0.288
Urine sodium output	0.4199	0.227
Net sodium balance	-0.0263	0.943
Age vs.:		
Water intake by drinking	0.4825	0.158
Total water Intake	0.4722	0.168
Urine water output	0.4650	0.176
Stool water output	0.2069	0.566
Net water balance	0.3383	0.339
Sodium intake	0.2265	0.529
Urine sodium output	0.5539	0.097
Net sodium balance	-0.3798	0.279

Table 14. Correlation statistics relating blood pressure variables to water and sodium input and output.

Variables	r	P value
SBP vrs:		
Total water intake	0.489	0.151
Urine water	0.226	0.531
Net water balance	0.595	0.054
Total sodium intake	0.296	0.406
Urine sodium	0.339	0.338
Net sodium balance	-0.028	0.939
DBP vrs :		
Total water intake	0.744	0.014
Urine water	0.460	0.181
Net water balance	0.798	0.006
Total sodium intake	0.211	0.558
Urine sodium	0.482	0.158
Net sodium balance	-0.313	0.379

Table 15. Correlation statistics for mean daily temperature (MDT) and mean relative humidity (MDrH) against water and sodium intakes and outputs.

Variables	r	P value
MDT vs.:		
Total water intake	0.187	0.606
urine water	-0.081	0.824
Net water loss or gain	0.099	0.786
Total sodium intake	0.170	0.638
Urine sodium	-0.16	0.658
Net sodium balance	0.249	0.488
MDrH vs.:		
Total water intake	0.457	0.184
urine water	-0.258	0.472
Net water balance	0.370	0.293
Total sodium intake	0.338	0.339
Urine sodium	0.182	0.615
Net sodium balance	0.293	0.411

Table 16. Correlation statistics for water drank and sodium intake against water and sodium outputs in urine and as net balance.

Variables	r	P value
Water intake (drink) vs..		
Urine water output	0.8742	<0.001*
Net water balance	0.8288	0.003*
Urine sodium output	0.8232	0.003*
Net sodium balance	-0.5307	0.115
sodium intake vs..		
Urine water output	0.4564	0.185
Net water loss or gain	0.1232	0.735
Urine sodium output	0.6848	0.029*
Net sodium balance	0.4639	0.177
Total water intake	0.8984	0.001*

*** Asterisks show a significant correlation.**

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 Discussion

The results from this serial study have shown that there are significant differences in ABP between and within individual subjects. It is also evident that the mean ABP values show a steady rise with increasing age. Additionally, low PRA values have been obtained in the study population, as well as positive net salt balance status. Altogether, an indication is given of the effect of salt, an environmental stressor, on ABP, a physiologic variable.

The experiments were, however, limited by the small size of the sample population for each series and the use of different subject groups for the serial study. The reason for the use of a small sample populations size and different subject groups can be attributed to limited logistics as well as the declination of some selected subjects to participate. It is, therefore, difficult to make general statements on the entire population. Nevertheless, each experimental series provided some pertinent data which may contribute to the understanding of Na^+ balance and ABP in Ghana.

With regards to the blood pressures of the subjects in the first series, the significant inter-subject differences in ABP found, were indicative of the uniqueness of each individual subject. Furthermore, the variability of blood pressure within each subject over time confirms that it is not static. Thus, the observed trend in ABP shows that this physiologic variable does not only vary within the day, but also from day to day.

Notwithstanding the variability in individual ABP shown, using the WHO criteria on the mean ABP enabled the classification of individual subjects as normotensive or hypertensive. On this basis the group showed an incidence of hypertension of around 22.2%, that is, 10 out of 45 randomly selected subjects. In 1978, Ikeme and his

colleagues (Ikeme et al., 1978) found a 15% incidence of hypertension during a survey of the same area (Mamprobi) on a large sample population. Since the present sample population size is small and was obtained from patients who had attended clinic, no meaningful comparison can be made. However, it would be expected that a study population chosen from out-patients attending clinic in a community, where the awareness of the disease is on the increase, would show a higher incidence. This rate may therefore reflect self-selection by hospital attendance.

It was also evident from the serial measurements of individual blood pressures that single or spot measurements of ABP may not truly reflect the individual's ABP. The ABP, apart from showing circadian variability (Pickering, 1988) may also show day to day alterations in response to changing physiological and environmental factors. This changing trend in ABP was clearly shown by the variability plots (Figs. 3a, b. c, d & e). It was also observed that with repeated measurements ABP tended to fall. This falling trend could be attributed to a reduction in the pressor response or a decrease in apprehension as the subject became familiar to the technique or observer (Mitchell, 1994). An increased pressor response or apprehension is often associated with "white coat" hypertension (Mitchell, 1994).

The plots for the normotensive and hypertensive groupings (Figs. 3b and 3c) show that certain values of blood pressure, when compared to the average ABP, deviate from it. These deviations of ABP can be considered as either false-negatives, when the values are below the average, or false-positives when above it. The plots for two individuals, one hypertensive (Fig. 3d) and the other normotensive (Fig. 3e) further exemplify these "abnormal" ABP determinations. For such determinations the subject could be falsely classified if, on the whole, observations are very few or irregular. In the clinical setting such misclassifications could be avoided by doing repeated serial measurements and comparing them to cohort or peer ABP profiles (Carandente et al., 1984).

The steady rise in ABP with advancing age, in this study, is shown for both SBP (fig 2a) and DBP (fig 2b). This rise of ABP with age was, hitherto, thought of as physiological or, due to the aging process. However, recent epidemiological evidence has revealed it as a feature associated with "Westernised" populations, as well as a feature of some traditional populations that have adopted lifestyles and cultures of western societies (Akinkugbe and Ojo, 1969; McLaren, 1985). The rise in ABP with age has been known to be a feature that is common in societies with high salt intakes and which also tend to have high prevalence rates of hypertension (Dahl and Love, 1954). In contrast, some traditional and rural societies, have shown little or no rise in ABP with age (Page et al., 1976: Pobee et al., 1977), and often, such groups have shown low ABPs as well as low salt intakes. For the subjects in this study, the rise in ABP with age found as well as the high incidence of hypertension, even though attributable to hospital attendance, suggests a high salt intake.

The second serial study showed consistently low PRA values in all the subjects, whether normotensive or hypertensive. Although PRA values are laboratory specific and are, therefore, limited by individual laboratory standards, the low values obtained in this study were comparable to values obtained by Solomon et al. (1987) in normal subjects on a high salt diet. Such low PRA levels suggest a suppression of the RAS, which is indicative of an increased sensitivity of the system. This phenomenon is linked to an increased salt intake (Sagnella et al, 1990). Furthermore, in a recent experiment by Volpe et al. (1993), a high salt diet was shown not only to be associated with such low PRA, but also with low aldosterone and increased plasma ANP levels.

It has been shown, in black populations, that an inappropriately low level of PRA or renin secretion exist (Saunders, 1987). This has been attributed to a possible impairment of the renin secretory process (Fray et al., 1987). However, low renin secretion is thought to be a physiological response to an increased plasma sodium concentration as well as volume expansion (Tobian, 1960; Overbeck et al., 1981).

Even though, no such impairment in renin secretion has been established in this study, normal Ghanaians have been found to have relative higher total body water, plasma sodium and exchangeable body sodium than their Caucasian counterparts (Addae et al., 1978), which are suggestive of a volume expanded state and, for that matter, indicators of a suppressed RAS and low renin secretion.

Considering that the need to conserve body sodium and water is high in the tropical resident, the low PRA levels in this study may indicate that the subjects were responding physiologically to a volume expanded state emanating from a high sodium intake and its accumulation in the body fluids.

It is also known that some salt-sensitive individuals tend to have low PRA with salt loading (Simpson, 1990). Notwithstanding the fact that the subjects in this study were not investigated for their salt sensitivity, there is some evidence to show that blacks, are generally more salt sensitive, that is, show a greater increase in ABP with an increase in salt intake or loading (Pafrey et al., 1981). This salt sensitivity is not very different between the urban and the rural population (Mufunda et al., 1993). It is also known that salt sensitive individuals have kidneys that would excrete slowly any load of salt and, therefore, are likely to respond to any increase in salt intake with a corresponding increase in ABP (Simpson, 1990).

It can be seen, from the data on the balance study, that the subjects had, on average, a high sodium intake (295.2 ± 9.0 mmol). Between subjects, the variability in sodium intake was very significant ($P<0.001$), ranging from 245.8 to 321.8 mmol per subject. Since no salt was added to their food at table, the subjects may have varied their intake of sodium by varying the amount of food ingested. This is derived from the fact that they were expected to consume as much as they needed or pleased, of the free meals that was provided in reasonably adequate quantities, three times a day.

Furthermore, the subjects may have shown elevated salt taste thresholds. A phenomenon that can be attributed to habituation, which arises from the frequent use of foods high in salt or sodium. This practice of using, frequently, foods high in salt is common in "Westernised" or acculturated societies. In addition, the practice, involving the use of salt as a food preservative and also as a condiment in certain fermented staples, which is widespread in most traditional communities undergoing urbanisation, could lead individuals into taking large amounts of salt in excess of body requirements.

In this study, although some local staples and preserved foods were used, the prior tasting of the food by a selection of subjects at cooking, ensured the adequacy of salt in the food. Thus, such high sodium intakes, as observed in this study, can be ascribed to habituation in the subjects, and which may be attributed. to a physiologic demand or genetic adaptation.

The relationship between sodium intake and its output is clearly shown by the significant correlation ($r=0.685$; $P=0.029$) between sodium ingested and urine output. Compared to intake, the average amount of sodium excreted through the urine (220.9 mmol) shows the kidneys as the major route for its excretion. The amount, in this study, is comparatively higher than the mean value of 114 mmol observed by Badoe and Osafo (1971) in normal Ghanaian subjects restricted in a hospital ward and on hospital diet. Nevertheless, this high urine sodium output never exceeded 300 mmol even for intakes far above this value. Thus, it would seem that when the amount of sodium ingested was above 300 mmol per day, the output via the kidney did not increase further. This situation may occur in individuals with kidneys avidly retaining or slowly excreting sodium. A mechanism that is suitable for conserving ECF sodium. The value of 300 mmol may, therefore, be taken as the maximum limit for excreting sodium by the kidneys of the study group, so that any excess intake over this amount results in sodium retention. The sodium retained by the subjects over the period, may result in ECF volume expansion and, subsequently, elevate their ABP.

The average amount of sodium excreted in stool (16.2 mmol per day), is high considering that stool sodium levels can be negligible in normal individuals on normal diets (Wrong, 1991). It is not surprising that this amount is proportional to that ingested, as would be expected in subjects with limited maximum renal sodium excretion.

Since sweat sodium loss was not directly determined, it is very difficult to show how much sodium was lost in this study group via the dermal route. However, in the acclimatised individual, sodium lost in sweat is only 10 mmol/l (Tinckler, 1966). This amount, which is about 17 mmol assuming a sweat volume of 1.7 litres, when compared to the estimated average net gain or loss of 58.1 mmol per subject per day in this study (see table 8), yields a difference of about 40 mmol. Assuming metabolic balance, this would represent sodium retained in each subject for each day. Over the entire period, the total amount retained would be quite substantial (40 mmol by 10 days = 400 mmol). The tendency to retain sodium may be associated with normal ambulant subjects who consume large amounts of foods with "moderate" sodium levels, are acculturated or urbanised and less physically active with minimal sweating.

Such an excessive amount of sodium, when retained in the ECF, would increase body weight, since salt retention and its attendant water retention would cause an increase in ECF volume. However, failure to weigh subjects throughout the period in the study and, especially, at the end of the study, makes it difficult to make any generalisations on net sodium gain.

Nevertheless, ambulating under the tropical weather may cause an increased loss of sodium through the cutaneous route since the ambient temperatures are usually about or above the critical sweating level (Badoe, 1968). So that, if sweat loss is copious, as would be expected with minimal physical exertion under the heat, then sodium loss in

sweat could be high in proportion to the magnitude of sweat lost (Briggs, 1975). In view of the above argument, the amount of sodium lost in the sweat of subjects in this study may be higher than that established for the acclimatised individual, inspite of the fact that they still retained a substantial amount of it. With high sodium intake, the subjects were expected to maintain balance under weather conditions that were conducive for sweating, in the face of their renal excretory limitations.

With regards to water, the average total intake (3258.5 ± 102.7) was high throughout the period. This value is far higher than the 2500 ml suggested by Badoe (1968) as replacement for the tropical resident undergoing surgery. Despite the fact that the average daily temperature and humidity did not vary much during the period of study, they remained close to the critical sweating level for most of the day. This may account partly for the large amounts of water ingested over the period by the subjects. Furthermore, the high intake of sodium may be another reason for the high water intake. So that the highly significant correlation ($P<0.001$) between water intake and sodium intake, shown in this study, should be expected, as water intake is a normal physiological response to sodium intake to maintain the ECF osmolarity.

The average amount of water excreted in the urine was 1432 ml per subject per day, a value that compares favourably with that obtained from restricted subjects (1443 ml) by Badoe and Osafo (1971).

For respiratory and cutaneous water loss, the average value of 1703 ml estimated in the study group was also similar to the value of 1700 ml obtained by Badoe in 1968 and of 1710 ml by Elebute in 1969 in hospitalised residents. One would have expected the value in this study to be higher than those obtained by Badoe (1968) and Elebute (1969), since the subjects were ambulant.

The loss of water from the respiratory and cutaneous routes is obligatory and is, for the most part, dependent on temperature and humidity, and is not affected by the quantities lost in urine. The effect of physical exercise adds to this obligatory loss. Thus, since the estimated water loss by the respiratory and cutaneous routes were similar in the ambulant and in those subjects restricted in a hospital environment, it would seem that ambulation did not have much influence on water loss via these routes

The sodium retaining tendency, found in this study, may be attributed to an adaptational response to conserving salt and water, associated with and accentuated by a reduced salt excreting ability as well as an increased intake. This could lead to volume expansion that may result in the elevation of ABP. So that, in the tropical resident, unless the bias to retain sodium is controlled by a reduction in the diet or by treatment, the tendency for volume expansion and subsequent induction of elevated ABP could lead, in susceptible individuals, to hypertension. The increase in salt intake would serve as a risk factor for developing cardiovascular diseases.

Although other environmental factors may contribute to the pathophysiologic states involving high blood pressure, dietary salt holds a unique position in the expression of hypertension in many persons, including the tropical resident.

4.2 Conclusion

In this study, a group of subjects showed evidence of an age-related increase in ABP, a phenomenon that pertains to societies with a high salt intake. In a second group, considerably low PRA values were obtained. An observation known to signify a suppression of the RAS and also associated with an increased salt intake. Finally, a third group showed an increased sodium intake and a lowered maximum limit of its excretion.

Considering that under the tropical climate, there is most likely a tendency to increase salt intake and to decrease its excretion, the resulting expansion of the ECF volume and lowering of PRA, may culminate in the elevation of the ABP. It is conceivable that any improvements in economic status, as well as the accessibility, increased availability and affordability of salt in Ghana, would enable this environmental stressor to result in elevation of the ABP.

In view of the limitations in this study, it may be prudent to conclude that, the tropical resident, even though adapted physiologically to the hot, humid conditions of the tropics, may have his ABP modified by sodium and its homeostasis. Such modifications may result in elevation of the ABP. This could explain the observed increases in the prevalence of hypertension in Ghana and, therefore give validity to the hypothesis.

REFERENCES

ADDAE, S. and Addae, F. : Effects of acute temperature changes on renal function in residents of the tropics : I. Haemodynamics. *Ghana Med J,* 1970, **8:** 178-183.

ADDAE, S.K., Dakubu, S., Larmie, E.T., Boatin, R and Belcher, E.H. : Total body water, total exchangeable sodium and related variables in the Ghanaian. *Clin Sc Mol Med,* 1978, **54:** 477-79.

AKINKUGBE, O.O. : High blood pressure in the African. London, *Churchill Livingstone,* 1972.

AKINKUGBE, O.O : Renal function in the tropics (Editorial). *Ghana Med J,* 1974, **13:** 94-95.

AKINKUGBE, O.O and Ojo, O.M. : Arterial Pressures in rural and urban populations in Nigeria. *Br Med J,* 1969, **2:** 222-4.

AMES, R.S., Borkowski, A.J., Sicinski, A.M, et al. : Prolonged infusion of angiotensin II and norepinephrine and blood pressure, electrolyte balance and aldosterone and cortisol secretion in normal man and in cirrhosis with ascites. *J Clin Invest,* 1965. **44:** 1171-86.

ARTHUR, S.K., Nyarko, A.K., Asaku, J.Y., and Akyeampong, A.Y. : Estimation of nephron function in normal Ghanaians using the creatinine and lithium clearances (Abstract). *Acta Physiol Scand,* 1990, **139(suppl):** 591.

BADOE, E.A. : Pulmonary and cutaneous fluid losses in the Ghanaian in Korle-bu hospital. *Ghana Med J,* 1968, **6:** 59-63.

BADOE. E.A and Osafo. J.D. (1971). : The daily urinary volume, sodium and potassium in adult Ghanaians. *West Afr Med J,* **7**: 391.

BEARD, T.C., : Dietary salt and blood pressure. *Lancet,* 1994. **343:** 546.

BEARD, T.C., Cooke, H.M., Gray, W.R and Barge, R. : Randomised control trial of a no-added-sodium diet for mild hypertension. *Lancet,* 1982, **2(8296):** 455-8.

BLAUSTEIN, M.P and Hamlyn, J.M. : Role of a natriuretic factor in essential hypertension : A hypothesis. *Ann Intern Med,* 1983, **98(Pt. 2):** 785-92.

BOHR, D.F and Berecek, K.H. : Relevance of vascular structural and smooth muscle sensitivity changes in hypertension. *Aust NZ J Med,* 1976, **6(suppl 2):** 26-34.

BOYD, .W., Adamson, R.A., Arnold, M., James, V.H.T and Peart, W.S. : The role of angiotensin II in the control of aldosterone in man. *Clin Sc,* 1972, **42:** 91-104.

BOYD, G.W and Peart, W.S. : The relationship between angiotensin and aldosterone. *Adv Metab Dis,* 1971, **5:** 77-117.

BRODY, M.D and Johnson, A.K. : Role of the anteroventral third ventricle region in fluid and electrolyte balance, blood pressure regulation and hypertension. In: Martini, L and Ganong, W.F.(eds) : *Frontiers in neuroendocrinology.* New York, Raven Press, 1980. 249-92

BUCKLEY, J.P and Jandhyala, B.S. : Central cardiovascular effects of angiotensin. *Life Sc,* 1977, **20(9):** 1485-95.

BRIGGS, L.C. : Environment and human adaptation in the sahara. In: Damon A (ed). *Physiological Anthropology,* N.Y. Oxford University Press, 1975, p93-129.

CARANDENTE, F., Ahlgren, A and Halberg, F. : Mesor-hypertension: hints by chronobiologists. *Chronobiologia,* 1984, **1(3):** 189-203.

COLLINS, K.J. : Endocrine control of salt and water in hot conditions. *Fed Proc,* 1963 **22:** 716-22.

DAHL, L.K and Love, R.A. : Evidence for a relationship between sodium (chloride) and human essential hypertension. *Arch Intern Med,* 1954, **94:** 525-31.

DAHL, L.K., Knudsen, K.D., Heine, M., et al. : Effects of chronic excess salt ingestions. Genetic influence on the development of salt hypertension in parabiotic rats: Evidence for a humoral factor. *J Exp Med,* 1967, **126:** 687-99.

de BOLD, A.J., Borenstein, H.B., Veress, A.T and Sonnenberg, H. : A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci,* 1981, **28(1):** 89-94.

de WARDENER, H.E and MacGregor, G.A. : Dahl's hypothesis that a saliuretic substance may be responsible for a sustained rise in arterial pressure : its possible role in essential hypertension (Editorial), *Kidney Int,* 1980, **18:** 1-9.

de WARDENER, H.E., Mills, I.H., Clapham, W.F and Hayter, C.J. : Studies on the efferent mechanism of the sodium diuresis which follows the administration of intravenous saline in the dog. *Clin Sci Mol Med,* 1961, **21:** 249.

ELEBUTE, E.A. (1969). : Evaporative fluid loss in adult Nigerian males. *Brit J Surg,* **56(3):** 213-216.

ELLIOT, P. : Observational studies of salt and blood pressure. *Hypertension,* 1991. **17(suppl. 1):** 13-18.

ELMER, P.J., Grimm, R.H., Flack, J and Lang, B. : Dietary sodium reduction for hypertension reduction and treatment. *Hypertens,* 1991. **17(suppl. I):** I182-I189.
FISHER, R.A and Yates, F. : Statistical tables for biological, agricultural and medical research. *Oliver and Boyd Ltd*, 1949. Edinburgh and London. 3rd Ed.

FITZSIMONS, J.T. : Angiotensin and thirst. *Proc R Soc Med,* 1972, **64:** 1074.

FITZSIMONS, J.T. : Angiotensin stimulation of the central nervous system. *Rev Physiol Biochem Pharmacol*, 1980, **87:** 118-146.

FRAY, J.C.S and Russ, S.M. : Mechanism for low renin in blacks: studies in hypophysectomised rat models. *J Hum Hypertens,* 1990, **4:** 160-162.

FREIS, E.D. : Salt volume and the prevention of hypertension. *Circulation,* 1976, **53(4):** 589-95.

FROST, C.D., Law, M.R and Wald, N.J. : By how much does salt reduction lower blood pressure? II. Analysis of data from within populations. *Br Med J,* 1991. **302:** 815-818.

GAVRAS, H. : Possible mechanisms of sodium-dependent hypertension : volume expansion or vasoconstriction? *Clin Exp Hypertens [A],* 1982, **4(4-5):** 737-49.

GAVRAS, H. : How does salt raise blood pressure ? A hypothesis. *hypertension,* 1986, **8(1)** 83-8.

GLEIBERMANN, L. : Blood pressure and dietary salt in human hypertension. *Ecol Food Nutr,* 1973, **23:** 143-56.

GUIDELINES for the treatment of mild hypertension: Memorandum from a WHO/ISH meeting. *J Hypertens,* 1985, **4:** 383-86.

GUYTON, A.C. : Circulatory Physiology III. Arterial Pressure and Hypertension. *Saunders,* 1980, Philadelphia, P.A.

GUYTON, A.C. : Long term arterial pressure control: an analysis from animal experiments and computer and graphic models. *Am J Physiol.* 1990, **259:** R865-877.

GUYTON, A.C., Coleman, T.G., Bower, J.D and Granger, H.J. : Circulatory Control in hypertension. *Cir Res,* 1970, **26 suppl. II:** 135-47.

HALL, J.E. : Control of sodium excretion by angiotensin II : intrarenal mechanisms and blood pressure regulation. *Am J Physiol,* 1986, **45(5):** 1431-7.

HALL, J.E.., Granger, J.P., Hester, R.L and Montani, J.P. : Mechanisms of sodium balance in hypertension: role of pressure natriuresis and diuresis. *Hypertens,* 1986, **4(suppl 4):** S57-S65.

HALL, J.E., Guyton, A.C and Mizelle, H.L. : Role of the Renin- Angiotensin system in control of sodium excretion and arterial pressure. *Acta Physiol Scand,* 1990, **139(suppl 1591):** 48-62.

HENRY, J.P. : Salt, stress and hypertension. *Soc Sci Med,* 1988, **26:** 293-302.

HENRY, J.P., Gauer, O.H and Reeves, J.L. : Evidence of atrial location of receptors influencing urine flow. *Cir Res,* 1956, **4:** 91-4

HIERHOLZER, K and Stolte, H. : The proximal and distal tubular action of adrenal steroids on sodium reabsorption. *Nephron,* 1969, **6:** 188.

HOFBAUER, K.G., Dienemann, H., Forgiarini, P., Stalder, R and Wood, J.M. : Renal vascular effects of angiotensin II, arginine-vasopressin and bradykinin in rats: interactions with prostaglandins. *Gen Pharmacol,* 1983, **14(1):** 145-7.

HOLLENBERG, N.K. : The renin-angiotensin system and sodium homeostasis. *J Cardiovas Pharmacol,* 1984, **6(suppl 1):** s176-83.

HOLLENBERG, N.K., Epstein, M., Guttmann, R.D., Conroy, M., Basch, R.I and Merill, J.P. : Effect of sodium balance on intrarenal distribution of blood flow in normal man. *J App Physiol,* 1970, **28:** 312-7.

HORWITZ, W. (ed). : *Official methods of analysis of the Association of Analytical Chemists*. 1975. p223.

HOUSTON, M.C. : Sodium and hypertension. A review. *Arch Intern Med,* 1986, **146(1):** 179-85.

IKEME, A.C., Pole, D.J., Pobee, J.O.., Larbi, E., Blankson, J and Williams H. : Cardiovascular status and blood pressure in a population sample in Ghana - the Mamprobi survey. *Trop Geogr Med,* 1978, **30(3):** 313-29.

JAMES, G.D and Baher, P.T. : Human population biology and hypertension: Evolutionary and ecological aspects of blood pressure. In: Laragh, J.H & Brenner, B.M (eds). *Hypertension: Pathophysiology, diagnosis and management.* Vol. 1 ch. 10. Raven Press Ltd., NY, 1990.

LANG, R.E., ThÖlken, H., Ganten, D., Luft, F.C., Ruskoaho, H and Unger, T. : Atrial Natriuretic Peptide - a circulating hormone stimulated by volume loading. *Nature,* 1985, **314(6008):** 264-6.

LARAGH, J.H. and Resmick, L.M. : Recognising and treating two types of long term vasoconstriction in hypertension. *Kidney Int,* 1988, **34(suppl 25):** S162-S174.

LARAGH, J.H. : Renal and adrenal factors in hypertension: diagnostic approaches. *Bull N Y Acad Med,* 1969, **45:** 859-76.
LARAGH, J.H and Sealey, J.E. : The Renin-Angiotensin-Aldosterone hormonal system and regulation of sodium, potassium and blood pressure homeostasis. In: Orloff, J. and Berliner R.W. (eds). *Hand book of Physiology - Renal physiology.* Bethesda, Am Physiol Soc. 1973, 831-908.

LARAGH, J.H., Baer, L., Brunner, H.R., Buhler, F.R., Sealey, J.E and Vaughan, E.D Jr. : Renin -angiotensin and aldosterone system in pathogenesis and management of hypertensive vascular disease. *Am J Med,* 1972, **52:** 633-52.

LAU. Y.T., Cheng, H.S., Hsieh, C.C. : Sodium transport in hypertension (Review abstract). *Chinese J Physiol,* 1992, **35(3):** 241-156.

LAW, M.R., Frost, C.D and Wald, N.J. : By how much does dietary salt reduction lower blood pressure? I. Analysis of observational data among populations. *Br Med J,* 1991. **302:** 811-815.

LAW, M.R., Frost, C.D and Wald, N.J. : By how much does dietary salt reduction lower blood pressure? III. Analysis of data from trials of salt reduction. *Br Med J,* 1991. **302:** 819-824.

MENDELSSOHN, F.A. : A method for measurement of angiotensin I in tissues and its application to rat kidney. *Clin Sci Mol Med,* 1976, **51(2):** 111-25.

MacGREGOR, G.A. : Dietary salt and blood pressure. *Lancet,* 1994. **343:** 545.

McLAREN, D.S. : Salt and hypertension. *Medicine digest,* 1985, **II(10):** 4-8.

McQUARRIE, I., Thompson, W.H and Anderson, J.A. : Effects of excessive salt ingestion of sodium and potassium salts on carbohydrate metabolism and blood pressure in diabetic children. *J Nutr,* 1936, **11:** 77-101.

MITCHELL, A.R. :Salt, hypertension and renal disease: comparative medicine, models and real diseases. *Postgrad Med J,* 1994, **70:** 686-694.

MORGAN, T and Gilles, a. : Effect of reduction in salt intake in hypertension. *Am Heart J,* 1979, **97(6):** 811-2.

MTABAJI, J.P., Nara, Y and Yamori, Y. : The cardiac study in Tanzania: salt intake in the causation and treatment of hypertension. *J Hum Hypertens,* 1990, **4:** 80-1.

MUFUNDA, J., Fink, G.D and Sparks, H.V.Jr. : Blood pressure response to dietary salt in rural and urban African men (Abstract). *Ethnicity and Diseases,* 1993, **3(suppl):** 846-58.

MUNA, W.F.T. : Cardiovascula disorders in Africa. *World Health Statistics Quarterly,* 1993, **46(2):** 125-33.

MUNTZEL, M and Drueke, T : A comprehensive review of the salt and blood pressure relationship. *Am J Hypertens,* 1992. **5:** 1S-42S.

MURRAY, R.H., Luft, F.C., Block, R and Weyman, A.E. : Blood pressure responses to extremes of sodium intake in normal man. *Proc Soc Exp Biol Med,* 1978, **157:** 432-36.

NORMAN, R.A., Coleman, T.G., Wiley, T.C., Manning, R.D and Guyton, A.C. : Separate roles of sodium ion concentration and fluid volume in salt loading hypertension in sheep. *Am J Physiol,* 1975, **229:** 1068-72.

OLIVER, W.J., Cohen, E.L., Neel, J.V. : Blood Pressure, sodium intake and sodium related hormones in the Kanomoto indians, a "no salt" culture. *Circ,* 1975, **52:** 146-157.

OVERBECH, H.W., Ku, D.D., Rapp, J.P. : Sodium Pump activity in arteries of Dahl salt-sensitive rats. *Hypertension*, 1981, **3:** 306-312.

PAGE, L.B. : Epidemiologic evidence on the etiology of human hypertension and its possible prevention. *Am Heart J,* 1976, **91:** 527-34.

PARFREY, P.S., Markandu, N.D., Roulston, T.E., Jones, B.E., Jones, J.C., and MacGregor, G.A : Relationship between arterial pressure, dietary sodium intake, and renin system in essential hypertension. *Br Med J,* 1981, **238:** 94-7.

PICKERIING, G.W. : The nature of essential hypertension. London, *J & A churchill,* 1961.

PICKERING, T.G. : The influence of daily activity on ambulatory blood pressure. *Am Heart J,* 1988, **116(4):** 1141-5.

PICKERING, T.G., Harshfield, G.A and Laragh, J.H. : Ambulatory versus casual blood pressure in the diagnosis of hypertensive patients. *Clin Exp Hypertens [A],* 1985, **7(2-3):** 257-66.

POBEE, J.O.M. : The management of systemic hypertension. *Ghana Medical Journal,* 1976, **15(2):** 136-41.

POBEE, J.O.M., Larbi., E.B., Belcher, D.W., Wurapa, F.K and Dodu, S.R.A. : Blood pressure distribution in a rural Ghanaian population. *Tans R Soc Trop Med Hyg,* 1977, **71:** 66-72.

POSTON, L. : Endogenous sodium pump inhibitors: a role in essential hypertension ? *Clin Sc,* 1987, **72(6):** 647-55.

ROWELL, L.B. : Human circulation: Regulation during physical stress. N.Y. *Oxford University Press,* 1986. p2-10.

SAUNDERS, E. : Hypertension in blacks. *Med Clin N Am,* 1987, **71:** 1013-29.

SHUNYU, I and Huang Ti Nei Ching Su Wen. (100 - 200BC): Cited by Swales, J.D. *Sodium Metabolism in Disease,* Lloyd-Luke, London, 1975. p1.

SIMPSON, F.O. : Blood pressure and sodium intake. In: Laragh, J.H & Brenner, B.M (eds). *Hypertension: Pathophysiology, diagnosis and management.* Vol. 1 ch. 15. Raven Press Ltd., NY, 1990. p205-15.

STAMLER, J.: Dietary salt and blood pressure. *Ann NY Acad Sci,* 1993. **676:** 122-156.

STREETEN, D.H., Conn, J.W., Louis. L.H. Fajans, S.S, Seltzer, N.S., Johnson, R.W, Gitler, R.D. and Dube, A.H. : Secondary aldosteronism: metabolic and adrenonocortical responses of normal man to high environmental temperatures. *Metabolism,* 1966, **9:** 1071-92.

SWALES, J.D. : Dietary salt and blood pressure. : The role of meta-analysis. *J Hypertens,* 1991. **9(suppl. 6):** 5S47-S49.

SWALES, D. : Studies of salt intake in hypertension. What can epidemiology teach us? *Am J Hyperten,* 1990. **3:** 645-649.

TINCKLER, L.F. : Fluid and electrolyte observations in tropical surgical practice. *B Med J,* 1966, **5498:** 1263-7.

TOBIAN, L. : The protective effect of high potassium diets in Hypertension and the mechanism by which high-NaCl diets produce hypertension - A personal view. In: Laragh, J.H & Brenner, B.M (eds). *Hypertension: Pathophysiology, diagnosis and management.* Vol. 1 ch 4. Raven Press Ltd., NY, 1990.

TOBIAN, L. : Interrelationship of electrolytes, juxtaglomerular cells and hypertension. *Physiol Rev,* 1960, **40:** 280-312.

TOBIAN, L and Binion, J.T : Artery wall electrolytes in renal andDCA hypertension. *J Clin Invest,* 1954, **33:** 1407-14.

UUSITALO, A., Ahonen, J.P., Gorski, P., Tuomisto, M and Turjamaa, V. : Does the biorhythm of morningness and eveningness predict the pressure level?, *Ann Clin Res,* 1988, **20(suppl48):** 51-3.

VANDER, A.J and Lucian, J.R. : Effects of mercurial diuresis and acute sodium depletion on renin release in the dog. *Amer J Physiol,* 1967, **212:** 651-6.

VOLPE, M., Tritto, C., Deluca, N., Rubattu, S., Rao, M.A., Larenza, F., Mirante, A., Enea, I., Rendina, V., Mele, A.F., et al. : Abnormalities of sodium handling and of cardiovascular adaptations during high salt diets in patients with heart failure (Abstract). *Circ,* 1993, **88(4 Pt):**

WRONG, O. : Water and monovalent electrolytes. In Garrow, J.S and James , W.P.T (eds). *Human Nutrition and Dietetics.* Churchil Livingstone. ch. 10. p 146-161.

YALOW, R.S and Berson, S.A : Reaction of fish insulins with human insulin antiserums. Potential value in the treatment of insulin resistance. *New Eng J Med,* 1964, **270:** 1171-8.

ZACHARIAH, P.K., Cornelissen, G and Halberg, F. : Ambulatory cardiovascular monitoring of healthy adults in Rochester, Minnesota; chronobiologic assessment. *Prog Clin Biol Res,* 1990, **341A:** 243-54.

APPENDICES

Appendix A

PROFILES OF BLOOD PRESSURE AND PULSE VARIABLES FOR THE SUBJECTS IN SERIES I

Table A1
ID. - JAB. AGE - 42

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	152	120	95	82	152	118	93	80
02	160	130	94	76	160	132	93	74
03	156	110	89	78	160	116	92	80
04	164	122	87	70	168	120	85	68
05	160	119	90	76	159	118	87	71
06	158	128	83	68	156	122	93	68
07	160	110	79	64	162	108	81	68
08	170	103	82	71	160	106	80	67
09	152	128	95	78	152	120	90	75
10	163	128	92	75	173	121	86	69
11	162	116	87	73	161	124	89	71
12	154	119	90	76	160	116	89	76
13	154	118	90	76	153	118	90	76
14	153	126	93	76	151	123	96	82

Table A2
ID. - COF AGE. - 41

01	158	149	108	88	152	158	110	86
02	160	138	94	72	168	144	96	72
03	160	128	91	72	160	140	100	80
04	160	136	101	84	162	136	100	82
05	163	146	97	72	164	139	92	69
06	163	130	92	73	163	122	89	73
07	159	153	112	92	159	148	110	91
08	163	140	102	83	161	140	100	80
09	164	144	105	85	164	148	103	81
10	164	118	86	70	160	120	88	72
11	164	140	100	80	164	138	99	80
12	168	108	81	68	168	110	83	70
13	176	100	79	68	172	100	76	64
14	165	121	92	77	165	119	86	70

Table A3
ID. - JOB AGE. - 23

Day	First recording sys	map	dia	pul	Second recording sys	map	dia	pul
01	109	87	76	84	108	80	66	88
02	92	69	58	84	93	72	61	84
03	114	87	73	84	101	80	69	86
04	99	77	66	88	95	70	57	75
05	103	82	72	95	102	80	69	83
06	106	81	69	68	97	77	67	70
07	119	88	72	86	116	87	72	89
08	114	90	78	58	109	80	65	51
09	122	94	80	72	121	95	82	76
10	108	83	70	92	110	78	62	96
11	112	92	82	84	112	91	80	88
12	108	83	68	80	106	82	70	84
13	112	87	74	88	108	84	72	92
14	100	83	72	92	108	81	68	88

Table A4
ID. LAB AGE. - 28

01	121	97	81	92	132	99	83	87
02	134	103	88	92	128	100	86	88
03	115	92	81	80	124	98	85	85
04	120	87	71	80	112	83	89	80
05	117	96	86	82	116	91	78	89
06	124	91	75	82	117	90	76	80
07	104	83	72	76	104	87	78	76
08	125	95	80	84	119	96	84	84
09	122	95	82	86	121	91	76	83
10	119	88	72	82	120	89	74	91
11	121	94	81	82	123	96	83	88
12	108	87	77	84	112	87	75	83
13	113	87	74	84	105	86	76	76
14	124	92	76	82	121	91	76	82

Table A5
ID. - WON AGE. - 26

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	118	87	71	62	110	85	72	62
02	126	100	87	56	126	97	83	62
03	118	91	77	60	116	86	71	59
04	128	99	84	66	122	96	83	64
05	112	87	74	60	112	84	70	65
06	109	91	82	71	112	93	82	61
07	119	96	84	73	112	93	84	81
08	123	94	80	71	122	95	82	74
09	114	87	74	64	116	90	77	68
10	116	92	80	71	121	100	90	73
11	115	96	86	76	111	92	83	74
12	119	94	82	74	107	92	85	68
13	111	85	72	78	109	82	69	80
14	114	97	89	72	116	99	90	65

Table A6
ID. - AUD AGE. - 67

01	140	102	83	60	152	107	84	59
02	176	124	98	69	168	121	97	68
03	154	107	84	66	150	103	80	70
04	132	103	88	70	136	99	80	70
05	132	97	79	60	132	99	82	74
06	143	108	90	70	140	107	91	68
07	132	99	83	67	137	98	79	68
08	154	113	92	60	154	111	89	59
09	124	92	76	75	129	95	78	73
10	168	116	90	64	152	112	92	62
11	130	101	87	63	137	106	90	60
12	112	83	68	60	127	87	67	59
13	124	91	75	64	126	93	76	63
14	134	95	75	62	132	89	67	64

Table A7
ID. - ODL AGE. - 56

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	121	96	84	72	123	93	78	69
02	124	95	80	76	112	86	73	69
03	120	95	82	80	116	92	80	84
04	121	97	85	72	118	94	82	77
05	121	92	77	72	119	92	78	76
06	110	86	74	64	116	88	74	68
07	122	97	84	64	121	94	80	60
08	118	90	76	76	119	90	76	72
09	120	90	75	87	115	88	75	84
10	125	90	72	75	112	85	72	73
11	115	85	70	86	107	84	72	84
12	132	104	90	72	125	107	98	76
13	129	103	90	75	130	99	84	75
14	120	101	92	80	125	100	88	84

Table A8
ID. - EMA AGE. - 53

01	162	125	107	54	160	120	100	55
06	165	123	101	57	176	123	96	59
07	150	114	96	68	161	126	108	68
08	171	135	117	63	174	130	108	63
09	164	123	103	54	173	132	112	56
10	166	127	108	62	172	127	104	63
13	142	111	95	65	148	116	100	63
14	154	117	98	57	158	122	104	60
15	149	114	97	57	146	115	99	60
16	168	125	104	52	164	127	108	62
17	178	137	116	60	170	125	103	58
19	138	111	98	57	139	115	103	57
20	138	115	104	58	152	117	100	56
21	134	107	94	59	139	110	95	61

Table A9
ID. - JAD AGE. - 24

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	128	88	68	56	122	93	78	58
02	110	79	64	62	108	81	68	66
06	103	82	71	60	106	80	67	76
07	128	95	78	52	120	90	75	52
08	128	93	75	73	121	86	69	63
13	116	87	73	61	124	89	71	62
14	119	90	76	60	116	89	76	63
20	118	90	76	53	118	90	76	53
21	126	93	76	53	123	98	85	53
22	147	100	77	56	131	96	78	54
23	117	88	74	61	116	87	73	62
25	131	96	79	52	122	93	78	52
26	124	97	84	54	118	90	76	56
27	125	93	77	48	113	91	80	49

Table A10
ID. - ELF AGE. - 36

01	128	103	90	76	120	96	84	76
02	117	96	86	70	116	95	84	64
03	123	98	85	73	116	95	85	70
04	124	99	87	67	120	96	84	67
05	125	92	76	66	120	91	76	66
06	120	101	92	69	124	95	80	72
08	124	98	85	66	112	87	75	67
14	118	107	92	72	119	98	87	70
15	128	102	90	75	124	100	88	75
16	132	105	91	55	136	103	87	68
17	128	104	92	85	112	99	92	87
19	131	96	79	79	140	111	96	50
20	130	101	86	60	124	98	85	64
21	118	97	87	84	116	93	81	83

Table A11
ID. - SAQ AGE. 34

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	152	122	107	64	158	117	97	64
02	148	112	94	61	144	119	106	68
03	140	116	104	66	150	117	100	72
04	148	116	100	65	142	111	96	56
05	132	111	101	73	140	116	104	67
06	140	115	102	63	142	121	111	64
09	144	119	106	76	149	112	93	67
15	145	118	105	60	144	115	100	60
16	146	118	104	76	142	111	96	76
17	149	124	111	60	148	123	110	60
18	179	134	112	62	159	120	100	60
20	164	125	106	52	156	124	108	52
21	160	124	106	60	141	114	100	66
22	145	115	100	58	137	107	92	60

Table A12
ID. - JEB AGE. - 50

01	137	102	86	89	131	96	78	87
02	123	92	76	77	123	92	77	79
03	124	93	77	79	125	89	71	83
04	121	89	73	76	126	97	82	88
05	132	95	76	105	129	90	71	89
06	131	91	71	78	132	89	68	81
07	121	98	87	80	128	96	80	80
08	160	144	91	76	151	110	90	76
09	140	101	82	72	142	98	76	70
10	148	97	72	72	140	102	83	70
11	117	94	83	84	114	86	72	82
13	114	86	72	84	113	84	69	86
14	128	99	84	60	127	96	80	83
15	124	92	76	87	122	88	71	80

Table A13
ID. PEH AGE. 21

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	128	91	72	72	120	93	80	84
03	132	101	86	82	115	93	82	84
04	128	103	90	90	130	105	92	81
06	130	103	90	92	125	104	94	95
07	134	111	99	84	138	110	96	77
08	130	109	99	88	131	110	99	92
15	130	99	84	85	122	98	86	88
16	130	99	84	83	111	89	78	85
17	129	104	92	91	131	106	94	82
18	132	107	94	92	121	106	98	95
20	136	111	99	89	132	110	99	93
21	137	105	89	81	146	107	88	96
22	132	105	92	92	128	103	90	92
23	129	103	91	89	126	100	88	98

Table A14
ID. - ELS AGE. 23

01	120	94	81	81	119	92	79	78
03	124	97	84	93	114	85	70	98
04	110	89	78	80	114	98	90	84
06	126	97	82	74	128	101	87	82
12	120	91	77	76	116	92	80	86
13	115	90	78	90	121	93	79	87
19	132	103	88	80	130	103	90	89
20	116	93	81	73	119	89	74	79
21	122	95	81	93	116	89	76	98
22	104	85	76	77	116	88	74	84
23	122	95	82	74	115	90	78	82
25	103	78	66	73	100	81	72	112
26	100	83	75	82	100	77	65	74
27	108	91	83	70	98	75	64	82

Table A15
ID. - ABA AGE. - 45

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	122	86	86	64	120	83	65	68
02	131	100	84	78	120	97	85	77
07	107	77	62	62	113	78	60	63
08	127	94	78	64	121	89	73	64
09	119	92	78	66	120	89	73	67
13	139	104	86	72	142	107	89	75
20	133	99	82	74	131	97	80	70
21	137	105	89	94	137	107	92	92
22	138	94	72	64	132	97	80	68
23	133	108	96	85	128	104	92	84
24	127	94	77	70	122	93	78	71
26	120	91	76	116	116	96	86	111
27	127	95	79	69	122	95	81	90
28	110	91	82	93	112	89	77	87

Table A16
ID. - PAN AGE. - 24

01	112	82	67	72	105	75	60	68
02	108	75	59	65	97	70	56	59
03	100	77	57	66	97	70	56	68
04	100	76	64	78	98	79	69	76
08	98	71	57	65	96	71	59	74
09	97	72	60	60	92	65	51	56
15	99	77	60	72	102	73	59	68
16	110	87	76	86	105	82	70	88
17	99	74	61	61	95	74	63	65
18	103	77	64	70	105	79	66	72
19	104	77	58	75	98	70	56	77
21	98	71	57	76	104	80	68	72
22	98	79	70	74	108	82	69	79
23	101	74	60	76	96	72	60	71

Table A17
ID. - EPA AGE. - 27

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	100	75	63	63	108	79	64	67
02	109	83	70	52	101	80	70	60
03	112	83	72	55	104	83	72	54
07	104	83	72	72	114	87	74	60
09	104	80	68	55	100	80	70	52
10	115	89	76	63	112	87	74	56
14	118	92	79	67	116	90	77	63
15	109	85	73	69	106	85	75	56
16	108	85	74	44	111	91	81	48
17	110	90	80	71	100	81	72	65
18	121	89	73	55	107	80	67	63
20	106	83	71	58	107	83	71	65
21	122	93	73	69	110	87	76	67
22	112	87	75	59	120	88	72	63

Table A18
ID. - SAO AGE. - 54

01	184	133	108	103	212	143	109	101
04	172	119	93	93	178	122	93	58
05	183	128	101	98	179	132	109	94
06	176	120	92	93	169	113	86	86
07	170	121	96	81	168	118	93	91
08	170	110	80	86	168	109	80	78
11	168	117	92	87	176	119	90	84
12	173	114	84	92	170	113	84	90
13	164	109	82	80	155	107	83	76
14	163	105	76	78	161	112	88	73
15	154	104	79	84	149	105	83	68
18	138	99	79	84	132	95	76	81
19	152	105	82	52	152	105	82	40
20	148	109	89	61	148	108	88	62

Table A19
ID. - EAB AGE. - 54

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	211	145	112	53	172	124	100	52
02	144	119	106	48	164	125	105	40
03	168	125	104	56	144	115	101	57
04	140	113	100	57	136	112	100	58
05	148	115	99	48	147	113	96	52
06	139	106	90	49	140	101	81	50
07	166	124	103	56	152	123	108	56
08	148	116	100	58	153	120	104	58
09	138	105	89	55	128	95	79	56
10	133	100	084	59	129	98	82	58
11	144	119	107	56	152	120	104	56
12	148	120	106	52	160	120	100	56
13	129	105	93	56	131	102	88	56
18	136	107	92	52	134	105	90	52

Table A20
ID. - CON AGE. 75

01	164	113	88	72	170	115	88	68
02	146	101	78	64	150	102	78	60
03	140	96	74	68	140	99	78	68
04	166	118	94	68	160	126	109	69
05	172	119	93	67	161	115	92	66
06	161	111	86	68	156	109	85	67
09	171	118	92	72	163	117	94	70
10	141	116	104	74	156	119	100	71
11	173	120	93	67	161	112	87	68
12	184	127	99	67	178	125	98	57
16	137	96	76	72	134	94	74	71
17	155	116	97	67	152	111	91	66
18	151	106	84	67	145	106	87	70
19	128	93	76	66	132	99	82	66

Table A21
ID. MAT AGE. - 50

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	171	121	96	64	156	109	86	60
02	144	100	78	64	133	96	78	60
03	127	89	70	67	116	88	74	67
04	133	97	79	66	124	92	76	67
05	129	93	75	61	128	93	76	62
06	113	86	72	72	118	87	72	78
08	127	96	80	60	132	97	80	63
09	116	87	73	67	112	79	63	70
10	116	81	64	64	120	86	69	66
11	129	93	75	72	122	87	70	76
12	122	95	82	63	116	89	75	64
13	117	86	70	76	106	83	72	71
15	132	100	84	72	138	103	86	68
18	130	94	76	72	128	95	78	72

Table A22
ID. - GRK AGE. - 50

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	114	85	71	72	108	84	73	72
02	102	78	66	61	98	73	60	64
03	111	86	73	68	112	85	72	72
07	94	69	57	52	100	75	63	58
08	105	76	61	66	93	71	60	68
09	100	76	64	74	100	75	63	68
10	107	81	68	56	109	82	69	64
13	104	78	65	64	103	74	60	67
14	92	70	59	59	94	71	60	58
15	119	97	86	73	117	90	76	72
16	104	85	76	71	107	87	77	73
17	106	83	71	66	101	79	68	64
18	108	83	70	64	101	78	67	56
19	106	81	69	61	112	85	71	58

Table A23
ID. - AGB AGE. - 59

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	140	107	90	56	140	107	90	60
02	160	113	90	60	160	113	90	60
04	150	110	90	64	150	107	85	64
05	150	113	95	67	148	103	80	63
08	144	111	94	61	144	101	80	65
09	165	118	95	59	153	113	93	60
10	140	101	81	63	125	95	80	64
11	144	104	84	68	168	115	88	69
12	179	125	99	43	163	118	95	58
14	144	111	94	70	156	118	99	56
15	137	104	88	56	138	109	94	67
16	152	109	87	66	145	106	86	69
17	146	109	90	70	142	107	90	64
18	142	105	87	61	157	114	92	60

Table A24
ID. - ABK AGE. - 24

01	104	85	76	93	100	87	81	78
02	103	81	70	87	97	80	71	80
03	99	84	76	84	101	86	79	84
05	98	79	70	89	96	78	69	85
07	100	79	69	85	100	77	66	81
08	95	76	66	80	92	73	64	81
09	98	81	73	87	99	82	74	85
12	92	73	64	86	88	74	67	83
13	95	76	67	81	91	76	69	83
14	103	82	72	85	102	81	71	80
16	103	83	73	93	100	83	75	89
17	93	74	65	84	95	77	68	84
18	101	82	72	92	101	82	72	89
19	92	73	64	92	93	76	68	92

Table A25
ID. - ELG AGE. - 44

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	160	120	100	68	160	113	90	68
02	193	144	120	76	191	149	128	72
05	162	124	105	61	146	119	106	67
06	138	107	91	68	133	106	92	66
07	148	116	100	68	144	112	96	66
08	144	113	97	64	132	95	76	63
09	148	116	100	68	142	111	96	65
12	148	111	92	58	134	101	85	68
13	122	85	67	73	120	88	72	74
14	128	89	69	70	123	90	73	67
15	141	113	99	46	132	108	96	48
16	140	110	95	61	136	109	96	65
19	156	119	100	64	160	123	105	64
20	130	94	76	72	130	99	84	68

Table A26
ID. - EUA AGE. - 36_

01	188	142	119	72	182	130	104	76
02	196	146	115	78	180	130	105	78
03	195	148	125	72	192	143	119	74
04	181	130	104	72	171	126	104	72
07	139	104	87	80	148	113	96	81
08	132	105	91	65	136	101	84	66
09	137	102	84	67	122	98	86	58
10	140	107	91	61	142	108	91	65
11	136	104	88	70	141	108	92	70
12	139	102	84	76	144	112	97	92
17	150	111	92	68	148	109	90	64
22	156	113	92	84	146	113	96	80
28	150	109	88	72	152	108	86	76
29	152	116	98	80	148	113	96	76

Table A27
ID. - BEA AGE. - 26

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	124	91	75	74	121	86	69	70
02	123	92	76	53	119	88	73	68
03	110	83	69	77	111	84	70	76
06	120	87	71	74	118	86	70	72
07	118	90	76	74	128	89	70	72
08	127	93	76	77	113	82	66	77
09	125	92	76	76	120	90	75	68
13	108	84	72	80	117	89	75	69
14	120	89	73	83	116	87	72	80
15	121	96	83	66	116	87	72	70
19	120	91	76	69	111	85	72	73
20	124	91	75	73	124	90	73	74
21	125	98	84	65	124	95	80	67
22	114	89	76	68	117	90	76	73

Table A28
ID. - EDT AGE. - 42

01	154	125	110	60	150	126	114	60
05	150	107	86	60	156	111	88	60
06	150	110	90	68	146	107	88	64
07	110	83	70	64	120	92	78	60
08	115	84	69	68	102	82	72	67
09	112	82	67	67	104	73	57	66
10	116	87	72	67	111	82	67	61
13	123	99	87	68	128	97	81	60
14	128	99	84	56	124	97	84	54
15	140	109	94	60	148	109	90	60
16	134	103	87	67	140	109	93	66
20	132	95	77	60	140	101	81	59
21	127	99	85	59	120	99	88	59
22	124	93	77	65	126	93	76	61

Table A29
ID. - VIQ AGE. - 19

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	130	90	70	80	130	90	70	72
02	120	83	65	68	120	80	60	72
03	116	89	75	85	110	85	72	87
06	131	100	85	84	127	98	84	76
07	126	90	72	72	114	88	75	72
08	131	97	80	85	124	94	79	85
09	120	85	68	89	116	84	68	85
10	123	86	70	66	125	88	69	80
11	120	85	68	89	122	90	71	88
12	104	91	84	80	122	86	68	88
13	111	88	76	76	112	88	76	80
14	108	81	67	87	114	90	78	82
15	116	81	64	77	114	86	72	77
16	116	85	69	86	108	87	76	85

Table A30
ID. - ADQ AGE. - 28

01	120	87	70	76	120	87	70	72
02	120	93	80	80	120	93	80	84
03	100	73	60	64	100	73	60	64
04	116	84	68	80	113	83	68	78
07	119	92	79	68	104	87	78	80
08	124	91	75	80	120	89	73	86
09	108	79	65	87	106	76	61	85
10	120	91	76	85	110	81	66	80
11	112	84	70	56	111	80	64	85
14	112	85	72	83	104	78	65	84
15	110	83	69	87	100	76	64	91
16	102	79	68	82	107	79	65	53
17	113	88	76	91	112	89	77	91
18	125	92	76	84	112	85	71	90

Table 31
ID. - MEN AGE. - 43

	First recording				Second recording			
Day	sys	map	dia	pul	sys	map	dia	pul
01	135	115	105	68	130	114	106	72
02	164	127	108	72	158	123	106	72
03	162	144	108	80	152	120	104	76
08	140	113	100	80	138	113	100	76
09	156	116	96	76	146	111	94	76
10	160	125	108	80	152	121	106	76
11	150	118	102	83	155	121	104	88
12	131	111	101	89	133	111	100	85
17	124	98	85	76	123	102	92	80
18	138	105	88	74	135	106	92	72
19	140	107	91	80	140	104	86	84
23	128	110	101	76	129	108	98	70
24	133	106	93	91	132	103	89	88
25	152	128	116	80	140	119	108	76

Appendix B

1. Preparation of buffer A

The buffer was made with the following;

- Sodium Phosphate (50 mmol/l)
- Sodium chloride (154 mmol/l)
- Disodium EDTA (10 mmol/l)
- Neomycin (0.2 g/l)

The compounds were weighed appropriately and dissolved in double-distilled water with continual stirring. The volume was then made up to almost a litre. The pH was then titrated to 6.5 +/- 0.05 with hydrochloric acid (HCl) or sodium hydroxide (NaOH) as necessary, using a pH meter (Gallenkamp pH STICK KPHX 120-B), and the volume finally made up to 1 litre.

2. Preparation of assay buffer

A litre of 0.05 mM Tris/HCl buffer (pH,7.5) was prepared using Tris (Hydroxymethyl) amino methane (Mol.wt. 121.14). The weight of the base required (6.06 grams) and, working from the Henderson-Hasselbach equation (see below) the volume of acid required (3.3 ml) were obtained.

The statement of the Henderson-Hasselbach equation is shown below;

$$pH = pKa + \log \frac{[Base]}{[Acid]}$$ where [] denotes concentration.

Both Tris base and HCl were dissolved in double distilled water and the volume made up to almost a litre. The mixture was stirred until well dissolved. A small amount (3 gram)s of Bovine Serum Albumin (BSA) was gently sprinkled on the solution to facilitate easy dissolution on standing. The pH was then measured with the pH meter, and where appropriate, titrated with HCl or NaOH to obtain values of 7.5 + 0.05. The buffer thus prepared was finally made up to volume and stored at 4^{o}C for later use.

Appendix C

Table 1. Chart showing daily water intake by each subject. These were measured to the nearest 10 (units - ml).

ID	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY10
AME	675	1575	1350	1125	1350	900	540	900	970	450
SBS	2160	1545	1980	1980	1710	1450	1820	1740	1575	1125
NAA	4635	4440	5040	2850	3600	1710	1350	3150	3150	3570
MNC	2250	1575	1350	2550	1575	1875	1585	1350	1930	1350
SLL	2000	1400	1400	1600	1640	1350	1725	1575	2025	1125
WON	1680	1890	1750	2100	1680	1855	2350	1575	1610	1575
MAG	2025	1575	1800	2220	1350	2250	845	1350	1500	1350
PEN	1575	2010	2025	2540	1840	1860	1250	1350	1400	1920
JAB	1885	670	1010	2475	1650	2250	2700	1450	1200	1450
PAA	2540	2250	1575	1575	2175	1430	2250	1850	1800	1950
Mean SE	2142.5 319.2	1893 312.6	1928 359.9	2101.5 170.1	1857 207.5	2093 438.7	1641.5 213.9	1629 187.9	1716 188.7	1586.5 258.8

Table 2. Chart showing daily water input by each subject as a component of food (units - ml).

ID	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY10
AME	860	520	890	480	870	840	1210	980	1040	730
SBS	1010	570	1050	740	830	1130	1310	1240	1530	1020
NAA	1020	560	1180	710	830	1170	1260	1350	1180	1040
MNC	1100	570	1130	690	930	1070	1390	1160	1420	1110
SLL	940	620	1070	680	850	1180	1250	1000	1250	1010
WON	870	550	1090	630	870	1090	1130	1180	1260	1060
MAG	690	550	1080	520	830	980	1310	1110	1230	1020
PEN	990	580	1130	710	830	1150	1320	1170	1310	1100
JAB	880	770	1250	720	880	1160	1670	1200	1020	1100
PAA	840	780	890	920	1050	1090	1170	1500	1220	1020
Mean SE	920 36.9	607 29.1	1076 36.1	686 39.4	877 21.7	1076 33.3	1302 47.6	1189 48.6	1246 48.8	1021 34.5

Table 3. Chart showing daily sodium intake per subject as a component of food (mmol).

ID	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY10
AME	202.8	179.6	295.4	150.2	266.4	401.6	433.4	209.8	550.2	185.1
SBS	224.3	192.7	268.7	211.5	236.9	430.6	468.2	245.9	640.9	276.3
NAA	254.3	195.9	254.4	202.5	238.5	406.4	454.5	256.7	632.6	281.5
MNC	246.7	193.5	245.8	204.6	276.2	415.8	476.6	264.8	663.8	229.7
SLL	219.6	201.2	269.0	195.0	239.2	431.9	420.5	220.8	602.1	271.2
WON	168.3	180.7	284.1	182.9	249.7	413.8	450.4	243.1	602.7	273.7
MAG	144.6	197.7	254.3	170.4	237.2	362.8	469.0	246.1	610.4	280.3
PEN	223.2	229.5	286.2	209.4	228.4	419.2	455.2	195.2	643.4	300.2
JAB	230.2	208.6	284.8	239.6	184.8	204.1	176.0	195.2	454.5	280.2
PAA	227.3	229.5	240.8	276.9	148.8	199.6	158.4	202.2	574.2	298.8
Mean SE	211.3 9.9	200.9 5.5	268.4 6.0	204.3 11.2	230.6 11.9	347.6 32.6	396.2 38.6	228.0 8.3	597.5 19.2	267.7 11.0

Table 4. Chart showing daily water output as urine (ml)

ID	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7	DAY8	DAY9	DAY10
AME	760	1930	1110	1130	1280	1140	690	1010	1140	1190
SBS	1680	1960	890	1450	540	1360	1240	1700	1800	1360
NAA	2010	3200	3130	1480	2450	1360	1550	3230	2390	2960
MNC	960	1550	990	1120	1920	1330	740	1540	1130	1840
SLL	850	2280	1240	1530	1260	2020	1540	1250	1640	1460
WON	540	1450	1130	1750	1630	860	2100	1270	1610	1360
MAG	910	1580	1280	1910	1350	1400	1170	900	1790	2010
PEN	1040	1760	1240	1750	1540	1050	1650	1010	1540	1960
JAB	670	1010	980	1530	750	970	1000	1010	1250	1320
PAA	1340	1730	1430	1440	1250	1240	1460	1440	1200	1320
Mean SE	1076 147.3	1745 211.7	1342 205.1	1509 80.4	1397 172.1	1273 101.3	1314 137.5	1436 215.8	1549 124.5	1678 170.3

Table 5. Chart showing daily stool water output per subject (ml). Empty cells show no stool collections.

ID	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10
AME	9	140	130	140	100	170	110	200	90	50
SBS	30	200	130	90	180	240	320	110	180	140
NAA	110	160	100	110	120	110	190	110	140	140
MNC	40	110	180	50		310	330		120	210
SLL	30	80	170	190		50	180	90	70	90
WON	40	120	160	40	20	50	50	80	130	160
MAG	60	130	150	60	80	40	90	120	170	170
PEN	70	150	110	70	50	210	120	190	100	240
JAB			200		190	140		40	50	10
PAA		50		200	130	40	130	60	80	90
Mean SE	48.6 11.0	126.7 14.7	147.8 11.0	105.6 19.8	108.8 20.9	136 30.1	168.9 32.7	111.1 18.0	113 13.5	130 22.4

Table 6. Chart showing daily urinary sodium output per subject (mmol).

ID	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10
AME	155.3	247.6	133.3	180.9	160.4	153.8	144.8	191.6	273.8	237.9
SBS	191.2	264.6	174.4	169.4	109.5	287.6	206.3	145.7	362.0	247.2
NAA	161.4	259.7	398.1	220.7	237.4	256.2	190.2	390.8	293.3	289.7
MNC	164.9	311.1	206.6	152.4	216.7	226.0	126.2	321.1	260.9	333.8
SLL	124.4	273.1	207.9	184.4	135.9	324.2	134.0	169.1	361.1	230.0
MAG	133.6	201.3	235.0	187.3	232.8	138.5	212.2	166.0	358.1	273.5
WON	157.4	233.2	192.0	222.9	178.4	204.5	116.5	179.0	364.6	327.6
PEN	134.5	314.7	211.4	231.4	299.5	242.1	177.4	230.2	258.5	253.0
JAB	179.7	208.8	257.1	195.7	113.0	213.7	169.4	201.0	250.5	211.2
PAA	248.3	202.7	213.3	222.6	115.0	203.0	161.1	285.7	255.4	220.3
Mean SE	165.1 11.3	261.7 17.0	222.9 22.1	196.8 8.4	179.9 20.5	225.0 17.8	163.8 10.5	228.0 25.2	303.8 16.1	262.4 13.6

Table 7. Chart showing daily stool sodium output per subject (mmol). Empty cells show no stool collections.

ID	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10
AME	18.9	6.1	14.4	3.4	40.1	68.1	62.7	16.1	20.3	4.9
SBS	43.5	13.9	12.1	13.8	63.4	71.1	44.1	19.9	35.5	8.7
NAA	14.3	2.1	2.5	4.7	12.6	49.2	67.4	4.2	14.7	5.2
MNC	3.7	19.2	3.4	12.4		82.5	66.4		22.6	7.4
SLL	2.6	8.3	6.1	6.4		4.5	62.1	10.1	4.4	4.2
WON	2.1	7.1	6.7	8.8	5.4	12.1	12.5	8.8	11.7	15.2
MAG	17.2	5.4	3.9	2.4	4.4	6.7	13.4	5.5	27.4	7.5
PEN	11.2	5.3	9.9	3.6	1.5	27.5	9.7	4.4	30.1	8.6
JAB			10.1	2.5	6.1	2.4		12.2	16.5	10.8
PAA		3.7		12.6	2.2	1.4	2.5	21.0	10.3	18.1
Mean SE	14.2 4.8	7.9 1.8	7.7 1.4	7.1 1.4	17.0 8.0	32.6 10.2	37.9 9.3	11.1 2.2	19.4 3.1	9.1 1.4