

CATION CONTENT AND FLUXES IN RED CELLS OF  
NORMAL AND HYPERTENSIVE NIGERIANS.

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## DEDICATION

This thesis is dedicated to my husband and children who never believed that I was incapable of doing this research and thus gave me the courage and motivation, to persevere with this work to an end, in spite of numerous obstacles and difficulties encountered in its prosecution.

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PREFACE

These studies were performed in the department of Pharmacology and Therapeutics of the University of Ibadan, during the period October 1974 to August 1978.

The studies were begun during the tenure of a research fellowship in the department and continued after becoming a lecturer in the same department.

The laboratory bench work and clinical work which comprise the studies were done by me personally. The only exceptions were the determination of haemoglobin genotypes which was done by the haematology department, and the routine laboratory and radiological investigations on patients which were carried out by the hospital departments that normally have responsibility for these investigations. Standard abbreviations have been used in the thesis without previous definition. Other abbreviations are defined when they are first used.



CERTIFICATION

I certify that this work was carried out by Dr. (Mrs) A. Fadeke Aderounmu in the department of Pharmacology & Therapeutics, University of Ibadan, Ibadan, Nigeria during the period October 1974 to August 1978.



.....

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ABSTRACT

RED CELL SODIUM AND POTASSIUM CONTENT AND  
FLUXES IN NORMAL AND HYPERTENSIVE NIGERIANS

1. Erythrocyte sodium, potassium and water content have been determined in 908 Nigerians so as to:

1. establish normal values in Nigerians
2. compare values in Nigerians with known values in other blacks.
3. compare values in Nigerians with those of caucasians.

Red cell sodium, potassium and water were also determined in 7 caucasians who had been resident in Nigeria for periods varying from 6 months to 18 years.

The RBC sodium for Nigerians was considerably higher than those of caucasians, but the RBC potassium and water did not show any significant difference.

In the course of this work, the normal (control) subjects were grouped according to their genotypes. 3 genotypes were encountered: AA, AS and AC. There was no significant difference in the erythrocyte sodium, potassium and water of the individuals belonging to these 3 genotypes.

The results were also analysed for sex and age differences, and none was found.

Results of erythrocyte sodium, potassium and water from 3 siblings and their mother were also presented. These results differed from one another, suggesting that environmental factors are also important and probably just as potent determinants of RBC sodium, potassium and water content as are genetic factors.

2. 100 hypertensive subjects were studied. They were all newly diagnosed, mostly symptomless ambulant subjects who were attending the medical out-patient department of the University College Hospital, Ibadan. Their main pathological finding was systemic hypertension. They were followed up for periods varying from 18 months to 3½ years.

Investigations were performed on each patient which enabled their being grouped into hypertensives with normal renal function or hypertensives with abnormal renal function. Only those with normal renal function were included in the study.



The results obtained for the red cell sodium and potassium were significantly different from those of the controls. Their red cell water was also significantly different from that of the controls, but the difference in RBC water was not sufficient to account for the differences in the RBC sodium and potassium.

Here again, the RBC sodium and potassium were not related to age or sex. The RBC sodium and potassium content were in no way related to the mean blood pressures. Their values remained the same both before and during treatment. Adequate control and maintenance of the patients blood pressures within the normal range did not affect these two cations.

3. When red cells from controls and red cells from hypertensives were exposed to a high sodium load, the RBCs from hypertensive gained a lot more sodium and lost a lot more potassium than the RBCs from controls.

4. Normal red cells lost their potassium into isotonic sucrose media seven times as fast as red cells from hypertensive subjects.

5. Normal red cells have a slightly higher active sodium flux per hour than red cells from hypertensive subjects, but the difference is not statistically significant.

The rate constant for active sodium flux is higher for red cells of controls than for red cells of hypertensive subjects, but the correlation between intracellular sodium content and rate constant is not good ( $r = -0.43$ ). The rate constant for the red cells of the hypertensives is lower, but it correlates better with the red cell sodium ( $r = 0.53$ ).

CHAPTER I

REVIEW OF SODIUM AND POTASSIUM CONTENT OF  
ERYTHROCYTES, AND THE TRANSPORT OF THESE CATIONS ACROSS  
THE ERYTHROCYTE MEMBRANES, IN HEALTH AND DISEASE.

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CHAPTER I

REVIEW OF SODIUM AND POTASSIUM CONTENT OF  
ERYTHROCYTES, AND THE TRANSPORT OF THESE CATIONS ACROSS  
THE ERYTHROCYTE MEMBRANES, IN HEALTH AND DISEASE.

1. Introduction.
2. Historical background.
3. Anatomical considerations.
4. Physiological considerations.
5. Erythrocyte sodium and potassium content in caucasians in health and in disease.
6. Erythrocyte sodium and potassium content in Africans and other people of African descent in health and in disease.
7. Background to the present study and its objectives.

1. Introduction

(a) "That the physician of another age will be as familiar with the operations of the animal economy as he is at present with its anatomy, - I have not the least doubt ..... I will venture to predict that what the knowledge of anatomy at present is to the surgeon, in conducting his operations so will chemistry be to the physician in directing him generally, what to do and what to shun; and in short, in enabling him to wield his remedies with a certainty and precision of which in the present state of his knowledge he has not the most distant conception.

The words were those of William Prout, Goulstonian lecturer in 1831. His prophecy was a bold one to make over one hundred and fifty years ago, but it has been justified by the events of the last fifty years.

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Studies conducted in this medical school in recent years have shown that plasma electrolytes in healthy Nigerians may differ significantly in some respects from those of caucasians. Thus McFarlane, Akinkugbe, Adejuwon, Oforofuo, Onayemi, Longe, Ojo and Reddy (1970) reported that serum potassium levels in "normal" Nigerians were lower than in Europeans and Americans. Other studies have also shown that in uncomplicated essential hypertension, extracellular electrolyte abnormalities characterised by low serum potassium and sodium unassociated with acidosis or alkalosis can occur (Salako, 1971).

It is well known that potassium is essentially an intracellular ion, only about 2% of the total body potassium being extracellular. Even with sodium which is distributed mainly in the extracellular fluid, the fact that the intracellular compartment is so much bigger than the extracellular ensures that the total extracellular sodium is only a little greater than the intracellular. In studying, therefore, the body electrolytes in health and disease in any population, it is desirable to study the intracellular as well as the extracellular compartments. The intracellular compartment can be studied either in



tissue samples (e.g. muscle) or in isolated cell preparations (erythrocyte and leucocytes). Muscle biopsies have increased our understanding of the changes in intracellular cations in pathophysiological states characterised by plasma electrolyte disorders, but, to relate intracellular cations to cell water, it is necessary to measure the extracellular fluid which is usually done by using a marker assumed to be excluded from the cell. Such measurements depend upon adequate equilibration but it is often difficult to know when this has been achieved. For example, the inulin space is still increasing at 40 hours and never appears to equilibrate completely (Ling and Kromash, 1967). In disease states the distribution space of the marker may increase acutely so that it no longer measures the extracellular space. For example, bromide space approximates to total body water in patients on cardiac by-pass (Cleland, Pluth & Tauxe, 1966). Estimations of extracellular water have been made by using chloride as a marker and making certain assumptions about membrane function (Graham, Lamb and Linton, 1967) but this simplified method has not received independent confirmation and the validity of using chloride space is open to criticism (Barratt & Walser, 1968).

The use of an isolated cell preparation avoids most of these difficulties. The time required for equilibration is very brief and hence the time for passive trans-membrane 'leak' of the marker can also be brief. The isolated cell provides a more satisfactory model for estimation of intracellular cations since it allows results to be referred to any intracellular reference measurements without interference by extracellular change. The erythrocyte (RBC) and the leucocyte (WBC) are the most commonly used isolated cells for electrolyte studies. Of these, the RBC has the advantage that until recently, it was the more widely used, and it is available in far greater quantities than the WBC. However, it also has a number of disadvantages. It lacks a nucleus and the capacity for aerobic respiration and protein synthesis, and is therefore unrepresentative of most body cells. Sodium transport by erythrocytes is less than that by leucocytes (Patrick and Jones, 1974), and perhaps most important of all, erythrocytes possess a large and variable amount of haemoglobin which has an inverse relationship with erythrocyte potassium (Maizels, 1936). In spite of these limitations in its use, the ready

availability of erythrocytes was considered sufficiently attractive for using this model in a first step towards characterising the intracellular cations of Nigerians in health and disease.

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## Historical Background

One of the earliest analysts of biological tissue to become interested in inorganic biological material was Berzelius (1840). He was the first to notice the high proportion of 'ash' in muscle and to report that muscle contained sodium chloride, sodium lactate, potassium chloride, sodium phosphate and calcium phosphate. Shortly after that, Liebig (1847) noted that muscle ash was rich in potassium and poor in sodium while the fluid by which the muscle was bathed contained much sodium and little potassium. The fact that the principal cation in erythrocytes was potassium and that in plasma was sodium was clarified in the early part of the 20th century (Van Slyke, Wu and Mclean, 1923).

The permeability of the red cell membrane was intensively studied by Davson and Danielli (1938), Harris (1941) and Maizels (1949). Simultaneously, a similar kind of work was carried on on brain and nerves (Keynes, 1951) frog skin (Ussing, 1949) and frog muscle (Nastuk & Hodgkin, 1950). As a result of these studies, the simple view that all of sodium and chloride are extracellularly situated had to be modified as evidence showed that a much more complex

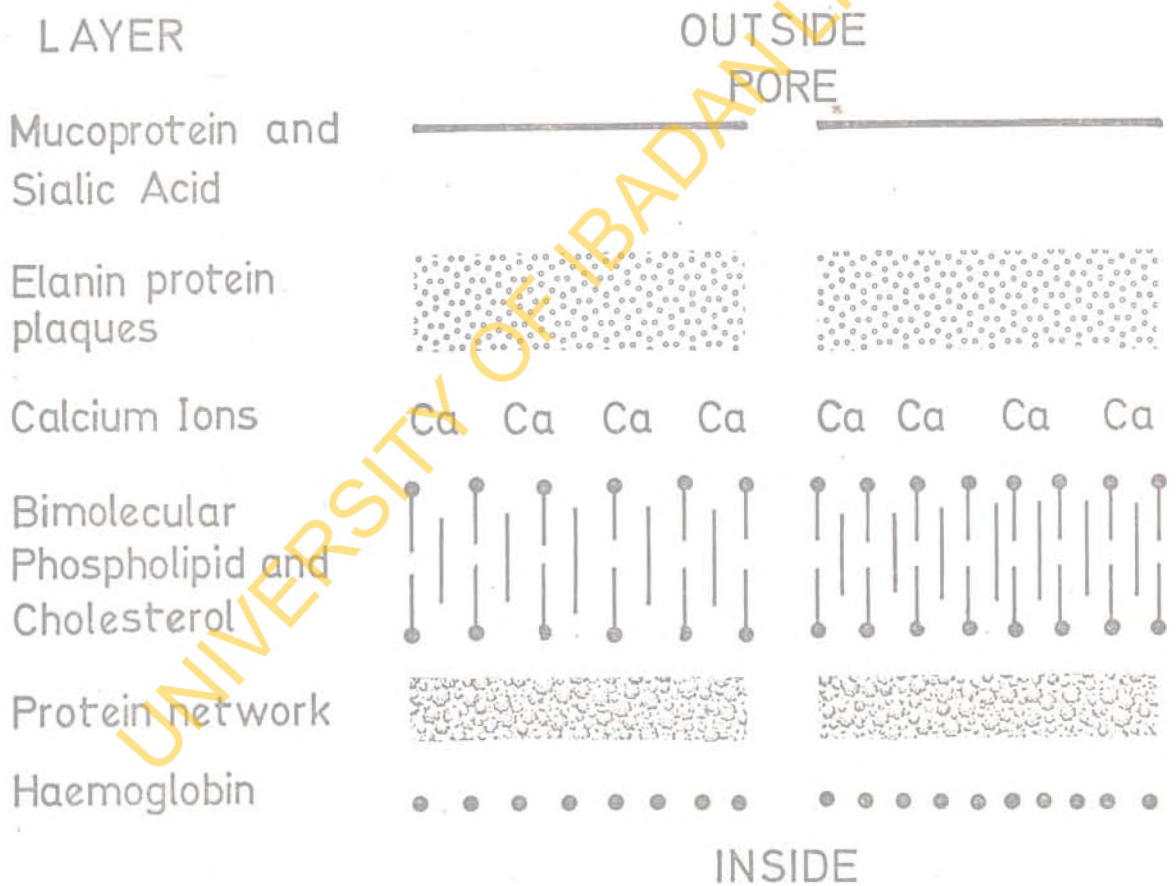


situation existed. The concept of cell membrane impermeability gave way to the view that exchanges take place continuously across a membrane which actively participates in the process by virtue of its own enzymes and metabolic processes.

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FIG.1

SCHEMATIC REPRESENTATION OF THE RED CELL MEMBRANE (PRANKERD, 1965).





### Anatomical Considerations

The electron microscope has revealed the detailed structure of the red blood cell membrane. Animal cell membranes are classified as:

- a. external (plasma) membrane, which is the type of membrane possessed by the RBC.
- b. internal (cytoplasmic ) membrane, possessed by bacteria.
- c. organelle membranes, e.g. mitochondrial membranes.

The structure of the red cell membrane is as shown in Figure 1 (Pranker, 1965). The RBC membrane consists of a bimolecular lipid layer bounded by protein (Davson and Danielli, 1936). The two lipid layers are bound together at their non-polar ends, the polar ends being turned away from each other, towards the protein layers. The lipid layer is penetrated by narrow pores about 0.4nm in radius. These pores allow penetration of small molecular weight particles and ions, but not large ones.

The mature RBC is one of the most highly specialised of cells. It is devoid of the usual

cytoplasmic organelles such as nucleus, mitochondria or ribosomes. Thus, it consists of little more than a membrane surrounding a solution of protein and electrolytes. More than 95% of the protein is haemoglobin. The remaining protein includes those enzymes required for energy production and for maintaining the haemoglobin in a functional reduced state.

The resting shape of the normal human RBC is that of a flattened bilaterally indented structure, a shape often referred to as a biconcave disc. In fixed stained blood smears, only the flattened surfaces are observed, therefore the appearance is circular, with an area of central pallor corresponding to the indented areas. The mean diameter of normal RBC after drying and staining was given as 7.2 $\mu$ m by Price-Jones (1933). Within the same preparation, there is, in addition, red cell heterogeneity - cells as small as 4.75 $\mu$ m and as large as 9.5 $\mu$ m in diameter being found (Price-Jones, 1933). Diameters measured in wet films are usually 0.8 - 1.4 $\mu$ m greater than those measured in dry films because methods involved in fixation and staining dehydrate the cells to some extent and

lead to reduction in size (Canham and Burton, 1968). The normal RBC volume as calculated from the packed cell volume (PCV) and the RBC count ranges from 80 to 96  $\mu\text{m}^3$  with a mean of 87  $\mu\text{m}^3$  (Baker, 1967). A greater mean value for the normal RBC volume (108  $\mu\text{m}^3$ ) has been obtained from measurements made from microscopy of hanging drop preparations (Canham & Burton, 1968). The surface area of the normal RBC is about 140  $\mu\text{m}^2$  (Canham & Burton, 1968) - considerably greater than that expected if its volume were distributed in a sphere.

Because of its disc shape, alterations in shape can be accomplished with relatively little change in surface area. If the RBC were spherical, considerable stretching of its membrane would be required to enable it to pass through small vessels.

How the RBC maintains its biconcave shape is incompletely understood. It has been suggested (Canham, 1970) that the cell simply assumes the shape that utilises the minimum energy of bending (least total curvature) since it reverts to this form within a fraction of a second after the deforming force is eliminated.



### Physiological Considerations

The RBC membrane has the property of selective permeability, hence it acts as a partial barrier to penetration of all solutes. In the case of non-polar substances, the rate of diffusion through the membrane is usually proportional to their lipid solubility. Polar solutes on the other hand, cross the membrane only at specialised sites or pores (Figure I).

Of the important polar substances, water and most anions (especially chloride and bicarbonate) diffuse freely and passively across the membrane. In contrast, the major monovalent cations, Na and K, require an energy-dependent transport mechanism - the sodium pump. This important transport mechanism maintains the internal osmotic environment and maintains the normal gradients between plasma and intracellular concentrations of Na and K.

Within the RBC, K is the predominant cation and Na is a relatively minor constituent, whereas in plasma, this relationship is reversed. The Na pump, apart from maintaining the internal osmotic environment of the RBC is also responsible for regulating the intracellular volume. If active transport is abolished, the cells accumulate Na

and water until a critical volume is reached, usually 1.5 times normal, and then haemolysis ensues.

The steady state cation concentration within the RBC is the result of an equilibrium between passive diffusion ('leak') and active transport (pump) (Tosteson & Hoffman, 1960). For Na the direction of the 'leak' is inward and that of the pump is outward. In contrast, K 'leaks' outward and is pumped inward.

Compartmentalisation of Na and K ions within the RBC: It has been shown that RBC sodium and potassium are distributed into at least two compartments within the red cell. The two compartments are:

1. An inner slowly exchanging compartment in which 25 to 35 % of sodium is located. Approximately 90% of potassium is also located in this inner compartment.
2. An outer more rapidly exchanging compartment in which 65 - 75% of sodium and only 10% of potassium are located (Beilin, Eyeions, Hatcher, Knight, Munro-Faure and Anderson, 1966).

Ion fluxes in RBC: Ion movements in RBC (or other cells) can generally be subdivided into three different types (Ussing, 1960). These are:

1. Active transport or flux which is the transfer of a substance against its electro-chemical gradient. The transfer takes place from a lower to a higher electrochemical potential and requires the expenditure of metabolic energy.

2. Passive transport or flux in which the ion moves passively down its electro-chemical gradient and does not normally require energy expenditure.

3. Exchange diffusion in which an ion exchanges with an identical ion, that is, sodium for sodium or potassium for potassium.

Net movements of ions can only occur through the first and second mechanisms, that is, through the pump and the 'leak' pathways. In red cells, sodium and potassium appear to be coupled in a single pump which operates to exchange specifically sodium inside for potassium outside (Glynn, 1956; Post and Jolly, 1957).

Active transport of sodium: It is now generally agreed that active transport of Na by human red blood cells is composed of three membrane pathways through



which Na is extruded from the cells (Hoffman & Kregenow, 1966). These different pathways have been shown to be pumps and can be classified as follows:

Pump 1a - a sodium outflux that is coupled to, and is dependent upon, the presence of potassium in the external medium. This flux is inhibited by cardiac glycosides and is unaffected by the presence or absence of external sodium. This is the well known and well characterised Na- K pump that derives its energy from ATP.

Pump 1b - a sodium outflux that requires the presence of sodium in the external medium. This flux is inhibited by cardiac glycosides and is unaffected by the presence or absence of external potassium.

Pump II - a sodium outflux that, like pump 1b has an obligatory requirement for external sodium and is independent of external potassium. Unlike pump 1b, it is insensitive to cardiac glycosides but is inhibited by ethacrynic acid.

Pumps 1a and 1b are referred to as glycoside sensitive, and pump II as glycoside insensitive. Pumps I and II together comprise 90-95% of the total sodium outflux of the cell. The remaining 5-10%

represents the passive outward leakage component.

Pump I makes up 70% ( $\frac{3}{4}$  pump 1a and  $\frac{1}{4}$  pump 1b) of the total sodium outflux compared with 20-25% for pump II. It thus follows that approximately 40% of the total sodium outflux is sodium-sensitive (Hoffman, 1966).

Other characteristics support the view that pumps I & II are entirely separate processes. These have been summarised as follows from a series of studies by Hoffman and his colleagues (Hoffman & Kregenow, 1966; Hoffman, 1962; Hoffman, 1966).

1. The two pumps differ in their concentration dependencies on the internal as well as the external concentration of sodium.
2. The two pumps are also linked to metabolism in different ways. Pump I is known to be driven by ATP and, during energy depletion (for example, continuous incubation at 37°C in the absence of added substrate), is completely inhibited after about 8 hours, correlating with the total disappearance of ATP from the incubating medium. However, pump II is still active at this time and does not begin to diminish in activity until after 14 hours or more.

The energy source for pump II is as yet unknown, but it does not appear to be ATP.

3. In ghosts made from fresh red blood cells, pump II is operative in circumstances in which pump I is absent.

4. Pump I can be reactivated in fresh ghosts by the addition of ATP or by incubation with adenosine without affecting pump II. On occasions, however, incubation with adenosine may resuscitate pump II but this is not consistent.

Active transport of potassium: Active outward transport of sodium for most cells including the red blood cell, requires the presence of potassium at the site towards which the sodium is transported. This suggests a coupling between the active transport of these two ions in opposite directions. Such coupling has, in fact, been shown to be the case (Glynn, 1956; Post and Jolly, (1957); (Tosteson and Hoffman, 1960).

Post and Jolly (1957) presented evidence that the active transport of potassium and sodium is rigidly linked in a ratio of 2 atoms of potassium to 3 atoms of sodium. Tosteson (1955) has estimated red cell active potassium influx as 1.9mmol/l/h and active

sodium influx as  $2.8\text{mmol/l/h}$ . The ratio is 2: 3 and is in good agreement with the data of Post and Jolly (1957).

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Erythrocyte Sodium and Potassium Content of Caucasians

(1) In Health: In the past five decades, RBC sodium and potassium have been extensively studied in normal caucasians (Maizels, 1936; Maizels, 1949; Keitel, Berman, Jones and MacLachlan, 1955; Czackes, Ullman, Ullman & Bar-Kochba, 1963; Funder and Wieth, 1966; Beilin, Knight, Munro-Faure, and Anderson, 1966).

Much is now known about RBC sodium and potassium content in health. Factors such as age, sex and phase of menstrual cycle which can possibly influence normal cation content have been elucidated by Beilin et al. (1966). However, in the literature, most of the series have been on small numbers of subjects. The series by Beilin et al. (1966) had the largest number of subjects - 144 in all.

From the studies on normal subjects above, certain data are now accepted about RBC cations in caucasians:

1. RBC sodium and potassium concentrations are relatively constant in individual subjects over lengths of time up to nine months (Beilin et al., 1966).
2. Variations in the RBC cations are greater from one



individual to another than within the same individual (Beilin et al., 1966)

3. RBC water content is relatively constant from day to day in the same subject, and it is also relatively constant from subject to subject (Beilin et al., 1966)

(2) In Disease: Alterations in intracellular cation content in disease have not been so extensively studied, even in caucasians. Studies have been done on patients with oedema (Kessler, Levy and Allen, 1961); on patients with anaemia (Maizels, 1936); in diabeti acidosis (Nichols & Nichols, 1953); in primary muscle disease (Dowben and Holley, 1959); in thyrotoxicosis (Smith and Samuel, 1970); and in essential hypertension (Edmondson, Hilton, Thomas, Patrick & Jones, 1975).

From the studies on caucasian subjects in disease states, it is now known that abnormalities in intracellular cations are associated with different diseases. These abnormalities occur in association with diseases like: uraemia (Smith, Welt and Czerwinski, 1967), body fluid disturbances (Kessler, Levy and Allen, 1961), thyrotoxicosis (Smith and Samuel, 1970) and essential hypertension (Edmondson. et al., 1975).

Erythrocyte sodium and Potassium Content of Africans and other blacks.

1. In Health: There is so far, no large series on cation content and transport in the erythrocytes of normal negroes. Available data are on small numbers of subjects, most of whom were controls used in studies on RBC cation content and transport in sickle cell anaemia (Tosteson, Carlsen and Dunham, 1955; Van Eps, Schouten, Sloof and Val Denden, 1971; Kurantsin-Mills, Kudo and Addae, 1974). These normal negroes have RBC cations and transport that are different from those of caucasians.

2. In Disease: Available data on negro subjects in disease are few. They are mainly on subjects with sickle cell (SS) disease (Tosteson, 1955; Tosteson, Carlsen and Dunham, 1955; Kurantsin-Mills, Kudo and Addae, 1974); Sickle cell trait (AS) (Tosteson, Carlsen and Dunham, 1955); and hereditary sodium transport defect (Balfe, Cole, Smith, Graham and Welt, 1968).

Background to this study and its objectives.

Macfarlane, Akinkugbe, Adejuwon, Oforofud, Onayemi, Longe, Ojo and Reddy (1970), working on serum electrolytes in various groups of healthy Nigerians and in a large hospital population (1,000 subjects), found serum K to be lower than for corresponding groups among caucasians. Serum Na on the other hand was found to have values comparable to those of adult caucasians.

From work done on serum electrolytes in hypertension in Nigerians, it has been found (Salako, 1971) that serum electrolytes in hypertensive patients differ from those of normal subjects - lower Na & K in the absence of any serious impairment of renal function. It is therefore possible that similar differences may exist between the RBC sodium and potassium in the two groups.

Furthermore, until about four decades ago, hypertension was believed to be a rarity in the African (Donnison, 1929; Vint, 1937). Recent studies, both hospital based and random studies among different populations throughout Africa, have shown, not only that hypertension is common, but also, apart from a



few exceptions like the Samura tribe in East Africa, there is a rise in blood pressure with age, in both sexes (Akinkugbe, 1972; Williams, 1941; Somers, 1960; Shaper and Saxton, 1969).

Epidemiological studies in several parts of Africa, the Carribean islands and among the black and white races of the United States of America, have shown that hypertension presents in black races at an earlier age and pursues a more severe course than in white races (Schrire, 1958; Akinkugbe, Solomon, French, Akinkugbe & Minear, 1976). More recently, it has also been found that low renin hypertension is more common in American blacks than in their white counterparts, and runs a more severe course (Laragh, Baer, Brunner, Buhler, Sealey & Vaughan, 1972). This new finding was given a fresh impetus to the search for the role of abnormalities of electrolyte metabolism in hypertensive disease in Africans.

Kurantsin-Mills et al., (1974) found in subjects with sickle cell haemoglobin SS disease, an impairment of red cell cation content and transport. The RBC Na was 40% higher in SS disease than in normals and ouabain sensitive active Na transport (pump 1a & b) was twice as fast as in normals. Passive efflux of

Na and K was also faster in haemoglobin SS states. Besides, they also found that normal caucasians living in Ghana had RBCs with 40 - 50% less Na than the RBCs of Ghananians with haemoglobin AA genotype. The RBC K was approximately the same in both groups.

Finally, malaria has also been known to cause changes in RBC membrane permeability to electrolytes and susceptibility to osmotic lysis (Dunn, 1968).

For the above reasons, it was decided to study RBC cation content and fluxes in a large number of normal and hypertensive Nigerians

1. to establish normal values.
2. to observe any abnormalities that may occur in hypertension
3. to observe any abnormalities that may occur in subjects with abnormal haemoglobin genotypes.

Much of the research presented here, may seem somewhat remote from practical medicine. Yet, if one reflects that some entirely academic work on the enzymes of intermediary metabolism is now being applied in medicine, for example, changes in serum levels of enzymes in muscular dystrophy (Thomson, Leybum and Walton, 1960), one would appreciate the need not to relegate fundamental research to the background in the pursuit of the more obviously practical.



CHAPTER II

GENERAL EXPERIMENTAL METHODS

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CHAPTER II

METHODS

1. Red Blood Cells - Sources
  - (a) Controls
    1. Blood donors
    2. Students and Staff.
    3. Relations and Friends.
  - (b) Hypertensive subjects.
2. Apparatus for Collecting Blood
  - (a) Blood Donor Samples.
  - (b) Other Controls.
  - (c) Hypertensive subjects.
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4. RBC Buffers.
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5. Equipment for Determination of Red Cell Water.
6. The Flame Photometer.
7. Standardisation of the Flame Photometer.
8. Coefficient of Variation for Individual Observations.

9. Procedure for Preparing RBC for Na and K determination.
10. Determination of Red Cell Water.
11. Determination of trapped plasma.
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13. Procedure for Preparing Red Blood Cells for Transport Studies.
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15. Hypertensive Subjects.
  1. Diagnosis.
  2. Blood Pressure Recording.
  3. Classification of Hypertensives.
  4. Initial Assessment.
  5. Routine Investigations on all hypertensives.
  6. Drug Regimen.
16. Follow up.
17. Analysis of results.

In the preceding chapter, the background to the studies which form the subject for this thesis and the objectives of the studies were presented. Relevant points in the anatomy and physiology of the erythrocyte in relation to its cation content and transport were also briefly discussed. In this chapter, the methods will be outlined and discussed.

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(1) Red Blood Cells - Sources

(a) Controls

Control subjects were drawn from three sources:

1. Healthy normotensive blood donors, whose systolic blood pressures did not exceed 140 mm Hg and whose diastolic blood pressures did not exceed 90 mm Hg, (WHO, 1962). Their ages ranged between 17 and 53 years, with a mean of  $27.9 \pm 0.15$  years. Of a total of 804 donors, 797 were males and 15 females. At the time of the study, these individuals were all at work and none was known to be taking any medication.
2. Medical students and staff of the departments of Anatomy, Physiology and Pharmacology, University of Ibadan. These were also healthy and normotensive, using the same criteria as above. Their ages ranged from 17 to 40 years, with a mean of  $23.3 \pm 5.2$  years. There were altogether 77 subjects in this group; 55 males and 22 females.
3. The remaining 27 subjects were drawn from among relatives and friends. They were also healthy and normotensive. Their ages ranged from 17 to 65 with a mean age of  $29.4 \pm 6.4$ . 26 were males and 1 was female.

(b) Hypertensive Subjects

These were drawn from the renal and hypertension clinic of the Medical Out-Patient Department, University College Hospital, Ibadan. Only newly diagnosed patients were included in the study. Their ages ranged from 23 years to 70 years with a mean age of  $48.8 \pm 1.4$  years. Of a total of 101 patients, 52 were men and 49 women. Ninety-three patients and symptomless hypertension detected on routine examination; 8 presented on account of symptoms related to hypertension. All were ambulant throughout the period of the study. Of the 8 who presented with symptoms, 3 had palpitations, 2 had hemiparesis, 2 had headaches and 1 had visual impairment.

(2) Apparatus for Collecting Blood

(a) Blood Donor Samples

In the blood donor clinic, 10 ml of blood was obtained from the antecubital vein into a heparinised bottle (LH/10, Stayne Laboratories) after 10 - 15 minutes venous compression. 2 ml of this was immediately transferred to a sequesterene bottle for genotype determination, the remaining being stored at room temperature ( $27^{\circ}\text{C}$ ) until centrifugation 1 hour later.

(b) Other Controls

10 ml of blood was obtained through a wide bore needle and treated as above. Most samples were obtained after minimal venous compression. Where the veins were not visible, venous compression lasted through the whole period of venipuncture, approximately two minutes. When the veins were visible, venous compression was maintained only until the needle was within the vein; it was released thereafter.

(c) Hypertensive subjects

Blood was obtained from hypertensive subjects in the same way as for (b) above.

(3) Preservation of Plasma

Plasma from each sample of blood was aspirated (after centrifugation), and stored frozen ( $-4^{\circ}\text{C}$ ) for 2 - 6 weeks in screw-capped bottles.

(4) RBC Buffers

1. Wash Solution

The red cell wash solution was made up of magnesium chloride 285 mmol/l buffered with Tris/HCl buffer to pH 7.4. The solution was prepared as follows:- 19.315 g of magnesium chloride (equivalent to 95 mmol) was weighed

and added to 2.423 g of Tris. The  $MgCl_2$  - Tris mixture was dissolved in 950 ml of deionised glass-distilled water. The resulting solution was then titrated against 0.1N HCl to pH 7.4 using an Isiebold pH meter at 20°C. The solution was then made up to 1 litre with deionised water. This constituted the 285 milli-osmol per litre buffered  $MgCl_2$ . It will subsequently be referred to as RBC wash solution.

The osmolality of the wash solution was determined for the first set of experiments by the freezing point depression method, using an Osmette A Automatic Osmometer (Precision Systems, U.S.A.).

## 2. Cold Storage Medium:

This was a high sodium having the following composition.

NaCl	110 mmol/l
$Na_2HPO_4$	25 mmol/l
HCl	2 mmol/l
$MgCl_2$	2 mmol/l
Adenosine	3.7 mmol/l
Glucose	10 mmol/l
Albumin	1 g/l

The composition of this medium is the same as that used by Post and Jolly (1957).



### 3. Incubation Medium

This was similar to the cold storage medium, except that the sodium chloride was completely replaced with potassium chloride in equimolar amounts. There were 2 lots of incubation media. Medium A for active transport contained all of the above for cold storage medium, but with KCl replacing NaCl. Medium B was similar to medium A, but in addition, it had ouabain 0.1 mmol/l to block active transport (pumps 1a and 1b).

### 4. Reagents for Permeability Studies

Isotonic sucrose solution was prepared by adding 103.058g sucrose (Analar quality) to deionised double distilled water. Tris 2.423g per litre was added to the mixture and the pH was adjusted to 7.4 by titrating the resulting solution with 0.1 N HCl. The solution was then made up to 1 litre with deionised water. This formed the isotonic sucrose solution used for the permeability studies.

### 5. Reagents for 'trapped plasma' determination

Evan's blue (T 1824) was obtained from Pharmacy Department, University College Hospital, Ibadan in 10 ml ampoules of 0.75%. This was diluted with normal saline to a concentration of 0.01% before being used for trapped plasma determination.

(5) Equipment for determination of RBC water

1. Petri dishes weighing between 70 and 90 g. The weights were accurately determined with a Mettler H 10W balance (Gallenkamp).
2. Thermostatically controlled oven.
3. Microhaematocrit centrifuge (Hawksley).
4. Glass pipettes.

(6) The flame Photometer

The Eel 150 Flame Photometer was used for the determination of Na and K. The flame photometer utilises the principle that alkali metals when raised to a sufficiently high temperature, will absorb energy from the source of heat, and be raised to an excited state in their atomic form. As these individual atoms cool, they will fall back to their original unexcited state, and re-emit their absorbed energy by way of radiation at specific wave-lengths some of which are in the visible region.

Therefore, if a mixture of the alkali metals Na and K and Li in solution are aspirated into a butane-air flame, in an aerosol form, they will after excitation by the flame, emit a number of discrete frequencies. These frequencies can be isolated by optical filters and individually

to fall on suitable photomultipliers, to be converted into electrical signals. The intensity of such electrical signals depends on the concentration of the alkali metals.

The electrical signals are read off a metre constructed to give direct measures of the concentrations of the alkali in milli-equivalent per litre.

(7) Standardisation of Flame Photometer

The Eel 150 was initially, and from time to time, calibrated against accurately known standards (Versatol).

(8) Co-efficient of Variation for Individual Observations

5 samples were prepared for routine Na and K determinations in the manner to be described. These samples were read on the Eel 150 on alternate days for 1 week, weekly for 4 weeks and monthly for 3 months.

Below are the results obtained for the mean, the standard deviation and the co-efficient of variation.

## M E A N

Sample	No of observations	Concentration in mmol/l ( $\pm$ SD)		Co-efficient of variation = SD/mean x 100	
		Na	K	Na	K
1	10	5.3 $\pm$ 0.246	72.3 $\pm$ 0.78	4.6	0.8
2	10	11.2 $\pm$ 0.42	110.2 $\pm$ 3.23	3.8	2.9
3	10	8.1 $\pm$ 0.53	84.8 $\pm$ 1.03	6.5	1.2
4	10	11.6 $\pm$ 0.37	93.5 $\pm$ 0.64	3.2	0.7
5	10	5.8 $\pm$ 0.126	95.3 $\pm$ 0.65	2.2	0.7

Thus, the coefficient of variation for potassium ranged from 0.7 to 2.9% while that for sodium ranged from 2.2. to 6.5%; indicating that the degree of accuracy for potassium determinations was higher than that of sodium.

From these results, it was shown that readings obtained on the same sample over a period of 4½ months did not vary by more than 7% for sodium or 3% for potassium. This is relevant, since collection of samples and subsequent flame photometry of these samples went on for a period of 3 years.



(9) Procedure for Preparing RBC for Na and K determination

Blood obtained as previously stated was placed in an M.S.E. centrifuge and spun for 15 minutes at 3000 revolutions per minute, at 20°C (room temperature). Plasma and the top-most layer of cells were aspirated and the cells were washed thrice with wash solution. Thorough washing was ensured by shaking the RBC in an automatic flask shaker (Gallenkamp). Washed cells were again re-packed by centrifugation and the supernatant was aspirated, following each wash.

After the third wash, the RBC were re-suspended in the buffer, enough buffer being added to give a haematocrit (PCV) of approximately 50%. The real PCV was then determined in triplicate using the microhaematocrit method.

Re-suspended cells were lysed by the addition of deionised water to make a 1 in 100 dilution. Each sample was prepared in triplicate. Red cell sodium and potassium were then determined by standard flame photometry. The value for each sample was an average of 3 readings. Samples were discarded if the minimum and maximum values of a triplicate reading

differed by more than 2%.

(10) Determination of Red Cell water

10 ml samples of blood were spun for 15 min. at 3000 revolutions per minute, the plasma was aspirated and the packed cells were washed thrice with the was solution as previously described. The PCV was determined.

1 ml samples of thoroughly packed and washed RBC were added to tared Petri dishes and weighed again with the dishes. The dishes (with the blood) were then placed in an oven at 100°C. In a preliminary set of experiments, samples were removed from the oven and weighed at intervals 8, 10, 12, 14, 16, 18 and 20 hours. It was found that the weight remained constant after 18 hours. Subsequently, the RBC were dried for 18 hours to obtain the dry weight.

The percentage (by weight) of water per 100 ml of RBC was then calculated. This constituted the RBC water %.

(11) Determination of trapped plasma

5 ml of whole blood was taken in heparinised bottles. The real PCV was determined. The blood was then spun and the plasma was aspirated. 2.5 ml

of 0.01% Evans blue dye was mixed with 1 ml of packed cells. The mixture was again spun and the supernatant was aspirated. Only those experiments in which the supernatant was haemoglobin-free were considered successful.

The optical density of the supernatant was then read off on a spectrophotometer (S.P. 600) at wavelength 540 u. The degree of dye dilution was then estimated, this being used in calculating the volume of distribution of the dye.

(12) RBC Passive Permeability Studies

Erythrocyte membrane permeability studies were done using the method described by Kurantsin-Mills et al (1974). Fresh blood was taken from subjects (normotensive and hypertensive) and washed three times as previously described. A volume of wash solution approximating to half the volume of the cell suspension was added to the suspension. The exact PCV was then determined.

The cell suspension was pipetted into isotonic sucrose (buffered with Tris-HCl to pH 7.4) to give a dilution of 1 in 10; the fraction of wash solution added together with the cells being taken into account. The actual volume of cells added was



determined by the PCV. The mixture was incubated at 25°C in a water bath with a gentle mechanical shaker.

Cells from the same individual were incubated for 10, 20, 30, 40, 50 and 60 min and also for 1, 2, 3 and 4 hours and the permeabilities observed over these periods. After each incubation, the cells were spun for 10 minutes, the sucrose supernatants were separated from the cells and the Na and K concentrations were determined from those supernatants as already described.

(13) Procedure for preparing RBC for Transport Studies:  
Sodium Loading of the Cells.

10 ml of cells were packed and the plasma and topmost layer of cells were discarded. 3 ml of cold storage medium (see page 33) was added to the red cells. The medium was thoroughly mixed with the red cells and the cells were stored at 4°C for seven to ten days. The cold storage medium was renewed every two days to provide fresh substrate for the cells and to keep the pH at  $7.4 \pm 0.2$ . Cold storage medium was prepared every 4 days and divided into 2 aliquots, one being used at once and the other frozen until needed 2 days later.



(14) Sodium Transport (Post and Jolly, 1957)

Stored cells were spun, the supernatant and the topmost layer of cells were removed, and the cells were thoroughly mixed with 5 ml of wash solution. Washed cells were spun and the supernatant aspirated. The cells were washed thrice. Finally, enough wash solution was added to the red cells to give a PCV of 45 to 50%. RBC Na and K of the loaded cells were then determined. The Na content of the supernatant was also determined and shown to be negligible.

0.2 ml aliquots of the loaded cells was added to 4.8 ml of the incubation medium. 4 experiments were set up for each sample, 2 for net transport, and 2 for passive transport. The PCV of each sample was determined after the 0.2 ml was mixed with the incubation medium.

Following this, the test tubes were shaken in a water bath at 37°C for 4 hours. The vibration produced in the water bath by an electrical stirrer ensured that cells and media were well mixed.

After incubation, the cell suspension was centrifuged and the cells were separated from the media. Only those experiments in which the media remained haemoglobin-free were considered successful.

Active Na efflux was estimated in the cells by measuring the decrease in RBC Na, and in the medium by measuring the increase in Na content of the medium. The Na determinations were carried out by standard flame photometry already described. The results were expressed as mmol/litre of erythrocytes, no attempt being made to correct for possible red cell volume or intracellular fluid changes (Post and Jolly, 1957).

(15) Hypertensive Subjects

These were all ambulant patients attending the renal and hypertension clinic of the Medical Out-Patient Department, University College Hospital, Ibadan. Only newly diagnosed patients were included in the study and they constituted a consecutive series. There were altogether 101 patients, 52 males, 49 females. Their ages varied from 23 to 70 years (mean age 48.8). They were followed up for periods varying from 1 to 3½ years.

1. Diagnosis

Only those individuals who were found to have a persistently elevated blood pressure, both in the erect and supine position, on repeated examination (at least twice) were labelled hypertensive. A blood pressure exceeding 140 systolic and 90 diastolic was

regarded as hypertensive WHO, (1962). Especially in younger age groups i.e. below 40 years. In patients over 55, the abnormal diastolic was set at over 100 mm Hg (Ayers, Slaughter, Smallwood, Taylor and Weitzman, 1973).

The blood pressure was measured on all occasions by the same individual, in the supine and in the erect positions. An accoson mercury sphygmomanometer with a cuff size of 23 cm by 13 cm was used on all patients.

## 2. Blood Pressure Recording:

The patient was made to lie down on the couch for 5 minutes before the supine blood pressure was recorded, he was then made to stand up for 3 minutes before taking the erect blood pressure.

## 3. Classification of hypertensives:

The hypertensives were grouped into two categories:

- (1) those with normal renal function.
- (2) those with impaired renal function.

Those with normal renal function:

In these patients, (1) mid-stream urine was examined at least twice and found to be albumin-free.

- (2) plasma creatinine was less than 2 mg per 100 ml.
- (3) blood urea was below 45 mg per 100 ml.
- (4) intra-venous pyelogram was normal.

Hypertensive patients with abnormal renal function:

These patients had:

- (1) Albuminuria detected twice in mid-stream urine examination.
- (2) Plasma creatinine above 2 mg per 100 ml.
- (3) Blood urea still in the normal range i.e. below 45 mg per 100 ml.
- (4) Normal or abnormal intra-venous pyelogram.

Those hypertensives with abnormal renal function were excluded from the study. Patients with overt fluid retention such as congestive cardiac failure or nephrotic syndrome were also excluded from the study. Patients with terminal renal failure were also excluded.

#### 4. Initial Assessment:

A new hypertensive patient was given a thorough physical examination, particular attention being paid

- to:-
- the cardiovascular system
  - the renal system
  - the fundi.

Each patient was assessed by me personally all the time.

The patient was subsequently classified as 'hypertensive with normal renal function', or 'hypertensive with abnormal renal function'. Observations were made in his notes, about his cardiac status as well as his optic fundi and all the other systems.



5. Routine Investigations on all hypertensives:

The following nine investigations were carried out on all hypertensives at first attendance:-

1. plasma electrolytes and urea.
2. liver function tests.
3. plasma creatinine and uric acid.
4. plasma calcium and phosphates.
5. blood sugar.
6. electrocardiogram.
7. microscopy and culture of mid-stream urine.
8. x-ray of chest.
9. Intravenous pyelogram.

At this initial assessment also, 10 ml of blood was taken from an ante-cubital vein, using a tourniquet to compress the vein only until the needle was in the vein as in the controls. 2 ml out of this was used for determining the haemoglobin genotype, and the remaining 8 ml for the erythrocyte and plasma Na and K determination.

6. Drug Regimen in Hypertensive Subjects:

So as to achieve uniformity in the treatment regime given to patients, the patients were classified into mild, moderate and severe hypertensives. This classification was based on the height of the diastolic blood pressure. Those patients whose diastolic

blood pressures were less than 110 mm Hg, were classified as mild hypertensives. Moderate hypertensives had diastolic blood pressures between 110 mm Hg and 120 mm Hg. Severe hypertensives had diastolic blood pressures above 120 mm Hg.

Patients in all 3 categories, if symptomless on first attendance; were put on diazepam 2 mg or 5 mg three times a day for the first 2 weeks, pending completion of investigations. On completing investigations, such patients were then put on definitive treatment.

Patients who, on first attendance, presented physical signs of impending heart failure, such as an apical third heart sound, were put on definitive therapy. In such cases, blood for all investigations was taken before starting treatment, and the initial 2 weeks of observation prior to therapy was omitted.

#### 1. Definitive Treatment:

Three drugs were mostly used as definitive treatment,

- they were:
1.  $\alpha$ -methyldopa
  2. debrisoquine
  3. propranolol.

The use of thiazide was purposely avoided so as to prevent any interference with the body's fluid and electrolyte balance. In a few patients who had side effects with methyldopa

and debrisoquine, hydroflumethiazide was added to the treatment so as to enable reduction in the doses of these drugs. These patients were subsequently excluded from the study.

The same observation holds for patients who were placed on digitalis or propranolol. They were excluded for the periods while they were on such drugs and for two weeks afterwards. Occasionally, diazepam was used as adjuvant to the definitive therapy in a few patients who were troubled with anxiety states. Digitalis was also occasionally prescribed to tide a few patients over periods of impending failure.

The mild hypertensives were usually easily controlled with methyldopa 250 mg two or three times daily or debrisoquine 10 or 20 mg twice or thrice a day.

The moderate hypertensives were also often controlled using the same drug regime as for the mild hypertensives. Sometimes, they needed larger doses of methyldopa or debrisoquine alone. Occasionally, they needed a combination of methyldopa plus debrisoquine or propranolol.

Severe hypertensives usually had combinations of large doses of methyldopa plus hydroflumethiazide or large doses of debrisoquine plus hydroflumethiazide. For example, methyldopa 1.5 g per day plus hydroflumethiazide 200 mg per day or debrisoquine 60 mg per day



plus hydroflumethiazide 200 mg per day.

16. Follow up:

The patients were seen at intervals of 2 to 8 weeks. At each visit, they were weighed and the urine was tested for albumin and sugar. The blood was measured in the manner already described and the prescription was renewed or modified as the need arose. The patient was also generally examined for any possible deterioration, or any concurrent illness.

At intervals during these follow-up visits, blood was also taken for RBC electrolytes as already described.

17. Analysis of Results:

Where appropriate, results obtained in this work were subjected to statistical analysis using the following methods:

1. Student's t test - This was used when comparing 2 unpaired data for statistical significance.
2. Paired t test - This was used when comparing data for statistical significance.
3. Analysis of Variance - This was used when comparing 3 or more data. In this case, when analysis showed a difference between the data, the data were then subjected to t test in twos to test for significant difference.



CHAPTER III

SODIUM AND POTASSIUM CONTENT OF ERYTHROCYTES.

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### CHAPTER III

#### Sodium and Potassium Content of Erythrocytes

1. Calculation of RBC sodium or Potassium Content
2. Calculation of Red Cell Water.
3. Calculation of Trapped Plasma.
4. Normotensive Controls - Results.
  1. Cation and Water content
  2. Breakdown of results into their various Hb genotypes.
5. Hypertensives
  1. Cation and water content
  2. Breakdown of results into their various Hb genotypes.
6. Caucasian Erythrocyte Cation Content.
7. Comments on Results so far Presented.
8. Influence of Age.
9. Genetic Factors.
10. Sex differences.
11. Plasma sodium.
12. Plasma Potassium.

The experimental procedures adopted for the determination of the red cell sodium, potassium and water content have been described under 'Methods'. From the experimental measurements made, the following calculations were used to obtain the values of the various parameters being determined.

1. Calculation of RBC Sodium or Potassium Content

RBC sodium (or K) =  $r \times \frac{50}{h}$  (this value is uncorrected for trapped plasma)

Where  $r$  = flame photometer reading, and

$h$  = value of artificial PCV.

Specimen Calculation.

Subject O.B. had an artificial PCV of 55%. His flame photometer reading for sodium was 15 mmol/l and for potassium 85 mmol/l. What is his RBC Na and K content?

RBC sodium content or potassium content =  $r \times d \times \frac{100}{h} \times \frac{1}{2}$

where  $r$  = flame photometer reading

$d$  = original dilution factor (which in all experiments was 100).

$h$  = value of artificial PCV

100 = PCV of 100%

200 = dilution factor for working standard  
for sodium and potassium in EEL 150  
flame photometer.

$$\begin{aligned} \text{RBC Na} &= r \times d \times \frac{100}{h} \times \frac{.1}{.200} \\ &= r \times \frac{50}{h} \\ &= 15 \times \frac{50}{55} = 13.6 \text{ mmol/l} \end{aligned}$$

Applying the correction for trapped plasma to RBC

Sodium

RBC Na corrected for trapped plasma

$$= \frac{\text{RBCNa} - (T \times \text{Plasma Na})}{1 - T} \quad (\text{where } T = \% \text{ trapped plasma} \\ = 0.03)$$

$$= \frac{13.6 - (0.03 \times 138)}{1 - T}$$

$$= \frac{9.46}{0.97}$$

$$= 9.75$$

Corrected value for RBC sodium = 9.8 mmol/l.



Calculation of Red Cell Water

2. Weight of Petri dish (empty) = 82.378g
- Weight of Petri dish (with blood) = 83.8657g
- ° Weight of blood = 1.4874g
- ° Wet weight of red cells =  $1.4874 \times \frac{PCV}{100}$
- Weight of RBC water = (wt. of blood  $\times \frac{PCV}{100}$ ) - dry weight of blood.
- Weight of dehydrated RBC = dry weight of blood.
- Weight of Petri dish (after drying) = 82.8156g
- ° Dry weight of blood = 0.4373g
- Weight of RBC water % =  $\frac{(\text{Wt. of RBC water})}{\text{wet wt. of RBC}} \times \text{dry wt. of blood} \times 100$
- $$= \frac{\text{wt. of blood} \times \frac{PCV}{100} \times \text{dry wt. of blood}}{\text{wt. of blood} \times \frac{PCV}{100}} \times 100$$
- $$= \frac{1.4874 \times \frac{PCV}{100} - 0.4373}{1.4874 \times \frac{PCV}{100}} \times 100$$
- PCV (mean of 3 determinations) = 90.3%
- ° Wt. of RBC water % =  $\frac{(1.4874 \times \frac{90.3}{100}) - 0.4373}{1.4874 \times \frac{90.3}{100}} \times 100$
- $$= \frac{(1.4874 \times .903) - 0.4373}{1.4874 \times .903} \times 100$$
- $$= \frac{0.9058 \times 100}{1.3431}$$
- $$= 67.4\%$$

### 3. Calculation of Trapped Plasma

1. At the beginning of each set of experiments, a calibration curve was drawn for various concentrations of the Evan's blue dye. The concentrations employed ranged from 0.005 mg/ml to 0.1mg/ml. The optical densities of the concentrations were read off the spectrophotometer, S.P. 600, at wavelength 540m. A graph (calibration curve) of optical density against concentration was then drawn this was a straight line within the range of concentrations used.

A typical set of figures from which a calibration curve was drawn was as follows:

<u>Concn<sup>n</sup> (mg/ml)</u>	<u>Optical density</u>
0.005	0.4
0.01	0.515
0.02	0.60
0.05	1.085
0.1	1.62

0.01% of Evan's blue dye was used for volume of distribution determination. 2.5ml of this solution of Evan's blue dye was thoroughly mixed with 1 ml of packed cells of known PCV. The mixture was spun in the centrifuge and the supernatant was decanted off.

The optical density of the supernatant was read off the spectrophotomer, and the corresponding concentration of the dye was then read off the calibration curve.

Specimen Calculation:

Trapped plasma (t) is equivalent to the degree of dilution of the 0.01% Evan's blue dye.

$$\text{Vol. of distribution} = \frac{\text{Quantity}}{\text{Concentration}}$$

Vol. of distribution of the dye = 2.5 + t (where 2.5 = Vol. of dye used for the experiment  
t = trapped plasma)

$$= 2.5 + t = \frac{\text{Quantity}}{\text{Concentration}}$$

$$= \frac{2.5 \times 0.01}{\text{Concentration}} \text{ (as read off the calibration curve)}$$

$$0.01 = \text{Concentration of dye in mg/ml)}$$

$$\frac{2.5 \times 0.01}{\text{Concentration}} = \frac{2.5 \times 0.01}{0.0099} = \frac{0.025}{0.0099}$$

$$2.5 + t = 2.5252 \text{ ml}$$

$$t = 0.025 \text{ ml}$$

$$\text{PCV of cells used} = 80\%$$

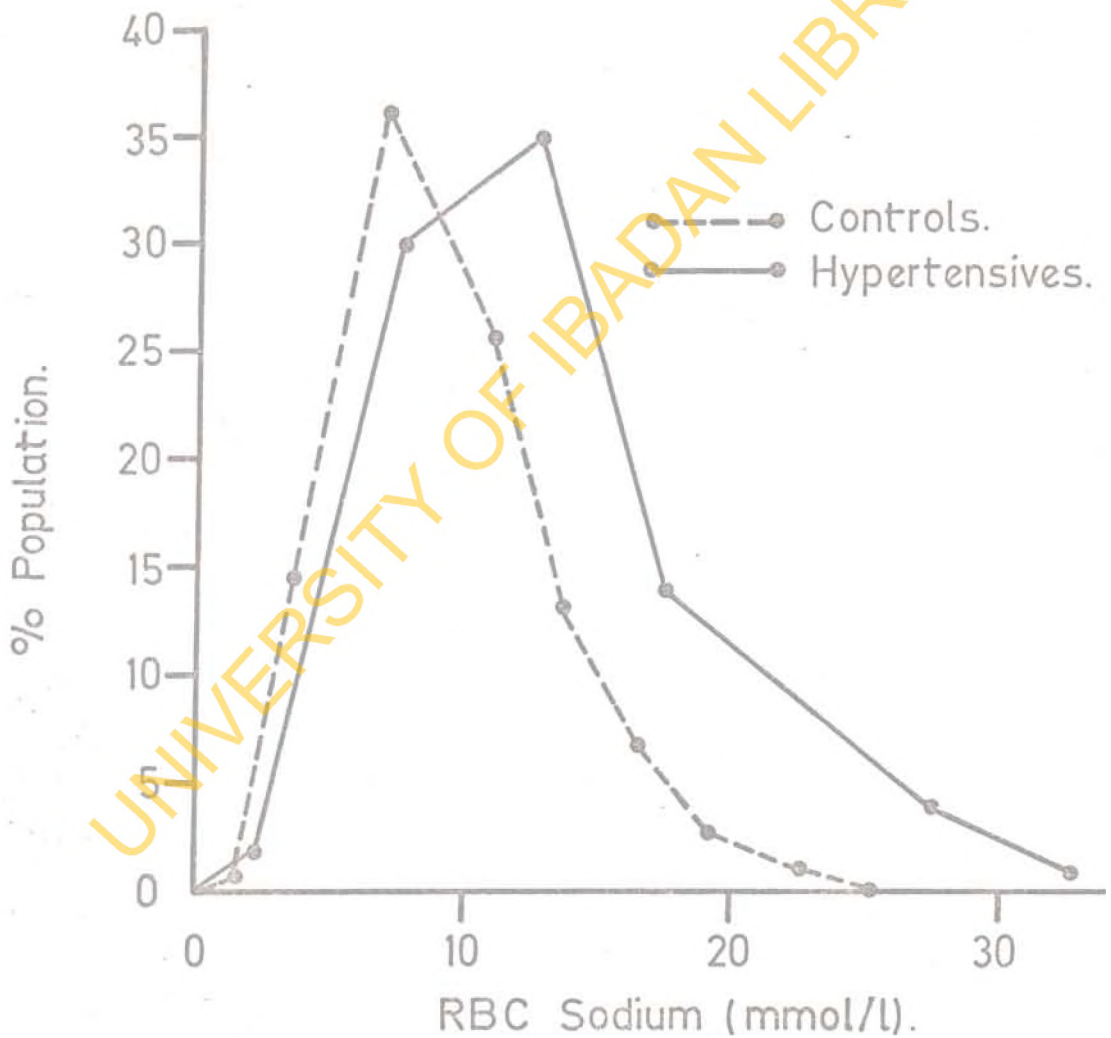
$$t \text{ in cells of } 80\% \text{ PCV} = 0.025 \text{ ml}$$

$$t \text{ in cells of } 100\% \text{ PCV} = \frac{0.025}{80} \times 100$$

$$= 0.031 \text{ ml.}$$

FIG.2

Distribution of erythrocyte sodium in normal and hypertensive Nigerian.





NORMOTENSIVE CONTROLS

4(i) Results:- Cation and Water Content. (Table: I, Figs. 2 & 3)

Erythrocyte sodium content for all normotensive controls irrespective of their haemoglobin genotype was  $9.7 \pm 0.13$  mmol/l per litre of red cells, the range was 2.4 to 28.2 mmol/l. Erythrocyte potassium was  $88.0 \pm 0.30$  mmol per litre of red cells, the range was 84.3 to 118.7 mmol/l. The total number of subjects was 908. RBC sodium results were corrected for trapped plasma.

The red cell water for normotensive controls irrespective of genotype was  $65.2 \pm 0.21\%$ . The range was 57.5 - 71.9%. The number of subjects was 71.

4(ii) Breakdown of results into various Hb. genotypes

AA genotypes: (TABLE I)

Normotensive control subjects with AA genotype had a mean erythrocyte sodium of  $9.6 \pm 0.15$  mmol per litre of red cells, the range was 2.4 to 24.7 mmol per litre of red cells. Erythrocyte potassium was  $87.9 \pm 0.32$  mmol per litre of red cell, the range was 68.1 to 118.7 mmol per

TABLE I

RED CELL SODIUM AND POTASSIUM IN 908 NORMOTENSIVE  
SUBJECTS WITH DIFFERENT HB. GENOTYPES

Subjects	Na <sup>+</sup> mmol/l	K <sup>+</sup> mmol/l	Red Cell Water %
AA Genotype	9.6 ± 0.15 [2.4-24.7; 633]	87.9 ± 0.32 [68.1-118.7; 633]	64.7 ± 0.51 [59.1-71.2; 46]
AS Genotype	9.7 ± 0.27 [3.1-23.3; 211]	88.7 ± 0.68 [70.0-113.0; 211]	65.6 ± 0.93 [56.4-71.7; 23]
AC Genotype	10.8 ± 0.76 [3.4-28.2; 50]	86.6 ± 1.28 [73.2-117.2; 50]	64.8 (-----, 1)
Undetermined Genotype	9.1 ± 1.01 [2.4-12.1; 14]	89.2 ± 4.0 [75.3-111.0; 14]	----- -----
All Controls irrespective of Hb genotype	9.7 ± 0.13 [2.4-28.2; 908]	88.0 ± 0.30 [68.1-118.7; 908]	65.2 ± 0.21 [56.4-71.7; 71]

In parentheses: range; number of subjects.

litre of red cells. There were altogether 633 subjects. Red cell water for this group was  $64.7 \pm 0.51\%$ , and the range was 59.1 - 71%. The number of subjects with AA genotype in whom red cell water was determined was 46.

#### AS genotypes

Normotensive control subjects with AS genotype had a mean erythrocyte sodium of  $9.7 \pm 0.27$  mmol per litre of red cells, the range was 3.1 to 23.3 mmol per litre of red cells. Mean erythrocyte potassium was  $88.7 \pm 0.68$  mmol per litre of red cell water, the range was 70.0 to 113.0 mmol per litre. The total number of subjects was 211. The red cell water for this group was  $65.6 \pm 0.93\%$ , the range was 56.4 to 71.7, and the number of subjects was 23.

#### AC genotypes:

Normotensive control subjects with AC genotype had erythrocyte sodium of  $10.8 \pm 0.76$  mmol per litre of red cells; the range was 3.1 to 23.3 mmol per litre of red cells. Their erythrocyte potassium was  $86.6 \pm 1.28$  mmol per litre of red cells. The range was 73.2 to 117.2. The total number of subjects was 50. The red cell water was 64.8%. It was determined in only one subject with AC genotype.

TABLE II

RED CELL SODIUM, POTASSIUM AND WATER CONTENT IN 100  
HYPERTENSIVE SUBJECTS WITH DIFFERENT Hb. GENOTYPES.

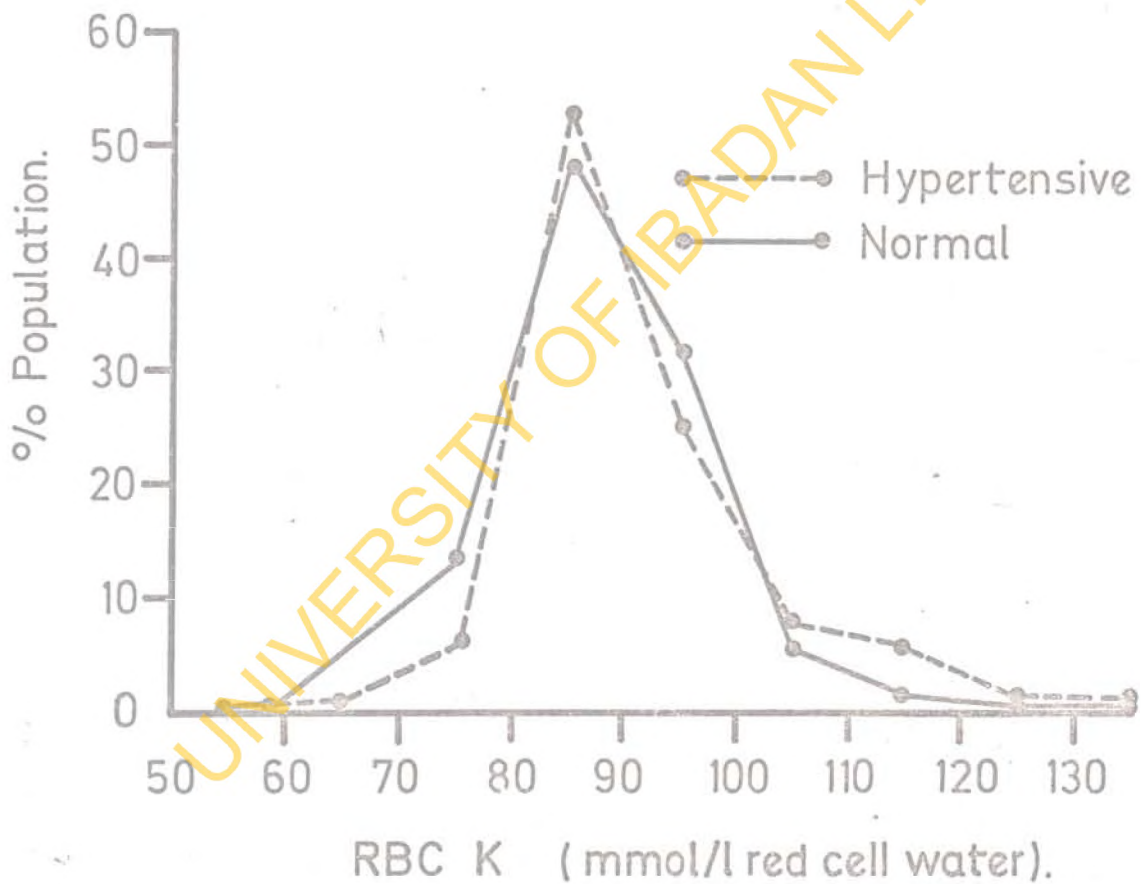
Subjects	Na <sup>+</sup> mmol/l	K <sup>+</sup> mmol/l	Red Cell Water %
AA Genotype	13.7 ± 0.91 [3.6-24.5; 45]	93.0 ± 1.80 [78.5-118.3; 45]	68.3 ± 0.6 [55.7-72.2; 41]
AS Genotype	16.9 ± 1.71 [9.0-31.3; 18]	93.2 ± 2.40 [82.1-115.1; 18]	67.9 ± 1.0 [65.4-72.6; 10]
AC Genotype	17.5 [-----; 1]	78.1 [-----; 1]	----- -----
Undetermined Genotype	11.4 ± 0.80 [4.4-24.3; 36]	87.7 ± 1.27 [67-106; 36]	69.4 ± 0.4 [64.8-73; 25]
All Hypertensives irrespective of Hb genotype	13.5 ± 0.59 [3.6-31.3; 100]	90.9 ± 1.04 [67.0-118.3; 100]	69.4 ± 0.36 [56.3-79; 77]

In parentheses: range; number of subjects.



FIG. 3

Distribution of RBC K in Normal and Hypertensives Nigerians.



5(2) Breakdown of results for hypertensives into their various Hb genotypes: (Table XXXIII & XXXIV).

↓  
AA genotypes:

Hypertensive subjects with AA genotype had a mean erythrocyte sodium of  $13.7 \pm 0.91$  mmol per litre of red cells, the range was 3.6 to 24.5 mmol per litre of red cells, and the number of subjects was 45. Their erythrocyte potassium was  $93.0 \pm 1.80$  mmol per litre of red cells. The range was 78.5 to 118.3 mmol per litre of red cells.

The red cell water for this group was  $68.3 \pm 0.6\%$ , the number of subjects was 41.

AS genotypes:

Hypertensive subjects with AS genotype had a mean erythrocyte sodium of  $16.9 \pm 1.71$  mmol per litre of red cells. The range was 9.0 to 31.3 mmol/l. Red cell potassium was  $93.2 \pm 2.40$  mmol per litre of red cells, the range was 82.1 to 115.1 mmol/l. The total number of subjects was 18.

Red cell water was  $67.9 \pm 1.0\%$ , the number of subjects was 10; the range was 65.4 to 72.6%.

TABLE III

CAUCASIANS

Name	Sex	RBC Na	RBC K	RBC Water	Length of Stay in Nigeria
1. N.D.	M	7.7	79.3	64.5	6 years
2. J.C.	M	8.6	80.9	62.1	3 months
3. A.R.	M	5.8	94.5	-	9 months
4. MMP	F	8.3	90.5	65.4	9 months
5. DMJ	F	8.1	100.4	-	18 years
6. M.M.	M	6.2	86.1	-	Unknown
7. F.N.	F	2.4	75.3	-	Unknown

N = 7

Mean RBC Na = 6.7	Mean RBC K = 86.7	Mean Red Cell Water = 6
S.D. = 2.2	S.D. = 9.0	S.D. = 1.7
S.E. = 0.83	S.E. = 3.4	S.E. = 0.98

AC genotype:

There was only one subject in this group. The erythrocyte sodium was 17.5 mmol per litre and the erythrocyte potassium was 78.1 mmol per litre. The red cell water was not determined in this individual.

Genotype Unknown: The genotype was not determined in 36 subjects. This group had a mean erythrocyte sodium of  $11.4 \pm 0.80$  mmol per litre of red cells, the range was 4.4 to 24.3 mmol per litre of red cells. Red cell potassium was  $87.7 \pm 1.37$  mmol per litre of red cells. The range was 67 - 106.8 mmol/l.

The red cell water was:-  $69.4 \pm 0.4\%$ .

6. Caucasian Erythrocyte Cation Content (Table III).

RBC sodium and potassium was determined in 7 normotensive caucasians. The red cell water was determined in 3 of these individuals. The results in these subjects were as shown below:

Red cell sodium  $6.7 \pm 0.83$  mmol/l (range 2.4 - 8.6)

Red cell potassium  $86.7 \pm 3.4$  mmol/l  
(range 75.3 - 100.4)

Red cell water 64% mmol/l (range 62.1 - 65.4).

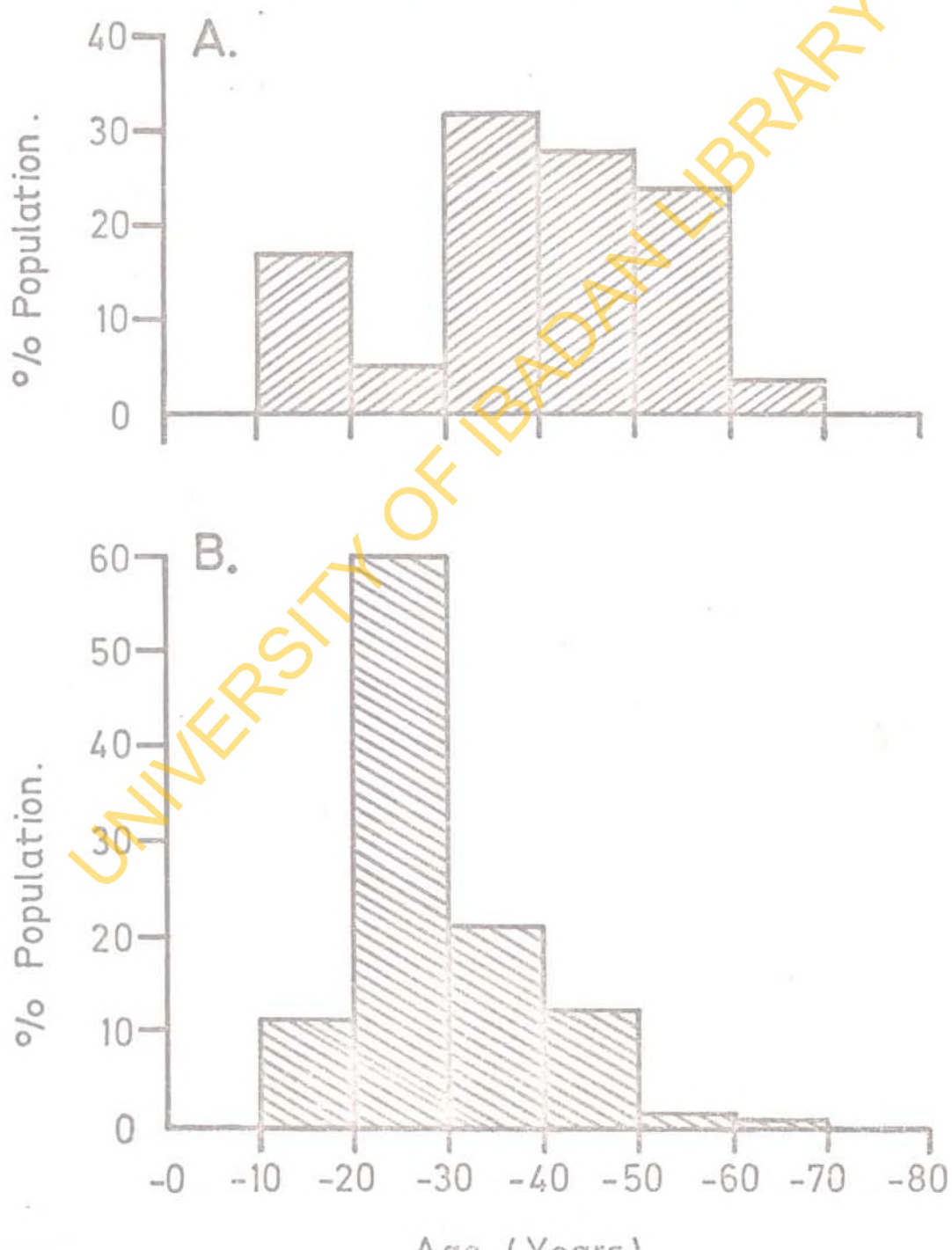


FIG. 4

Age distribution in normal (control) and hypertensive subjects.

A. HYPERTENSIVES.

B. NORMOTENSIVES.



Age Distribution (Fig. 4)

Controls: The ages of the blood donors ranged from 17 to 53 with a mean age of  $27.0 \pm 0.13$  years. The ages of the medical students and staff varied from 17 to 40 years, with a mean age of  $23.3 \pm 5.2$ . There were altogether 804 donors and 77 students and staff. Relations and friends formed the remaining 27 controls. Their ages ranged from 17 to 65 years with a mean age of  $29.4 \pm 6.4$  years.

Hypertensive subjects: The ages of the hypertensive subjects varied from 23 to 70 years with a mean age of  $48.8 \pm 1.4$  years.

From these data and from Figure 4, it was observed that the controls were much younger than the hypertensive group. However a correction for age was not felt necessary because neither RBC sodium nor RBC potassium seemed to vary very much with age, within the age limits studied in both the controls and hypertensives.

TABLE IV

COMPARISON OF THE RED CELL SODIUM AND POTASSIUM CONTENT IN  
100 HYPERTENSIVE SUBJECTS AND 908 NORMOTENSIVE SUBJECTS

Subjects	RBC Na mmol/l	RBC K mmol/l	RBC Water %
Normotensive Subjects	9.7 ± 0.13 (2.0-28.2; 908/	88.0 ± 0.30 (68.1-118.7; 908/	65.2 ± 0.21 (56.4-71.7; 717
Hypertensive Subjects	13.5 ± 0.59 (3.6-31.3; 100/	90.9 ± 1.04 (67.0-118.3; 100/	69.4 ± 0.36 (56.3-79; 77)
Difference between means	3.8 ± 0.60	2.9 ± 1.08	4.2 ± 0.42
t =	6.33	2.69	10.0
p value	< 0.001	< 0.01	< 0.001

In parentheses: range, number of subjects.

7. Comments on Results so far Presented:

From the results for red cell sodium, potassium and water contents obtained in the control and normotensive groups above (Table IV), the following observations were made:

1. The red cell sodium in hypertensive was higher than that in normotensive by approximately 39%. This difference was statistically significant ( $P < 0.001$ ).
2. The red cell potassium in hypertensives on the other hand was only minimally higher than those of normotensives. This difference was in fact only just statistically significant ( $P < 0.01$ ).
3. The red cell water in hypertensives was higher than that of normotensives ( $P < 0.001$ ) by 6.4%. This difference, though statistically significant was too small to account for the greater RBC sodium content of hypertensives over those of normotensives.

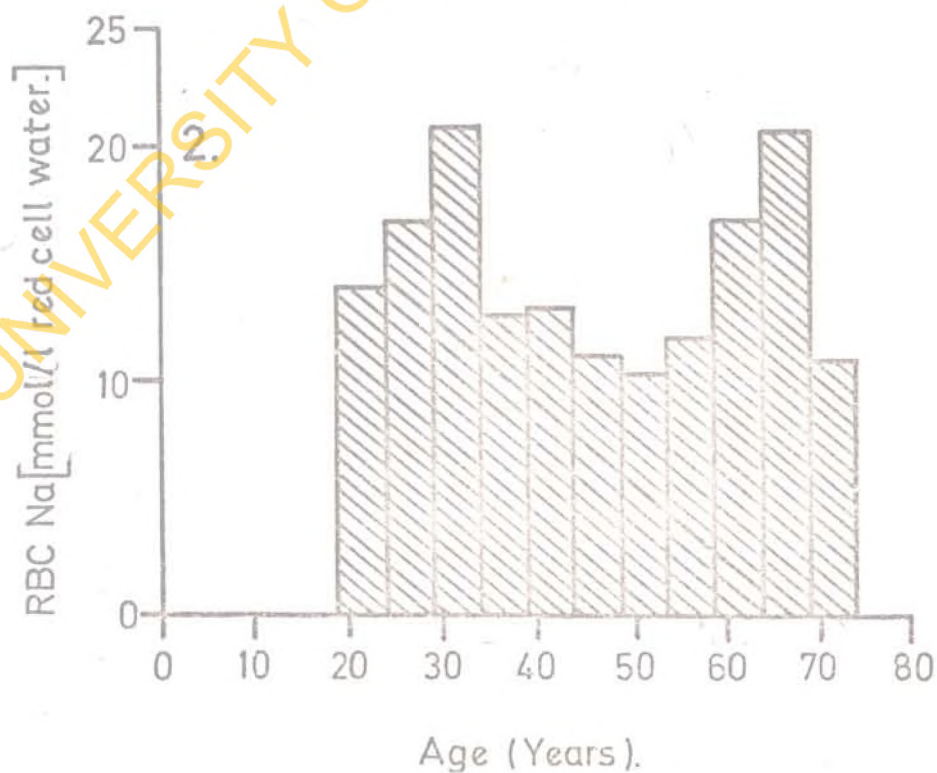
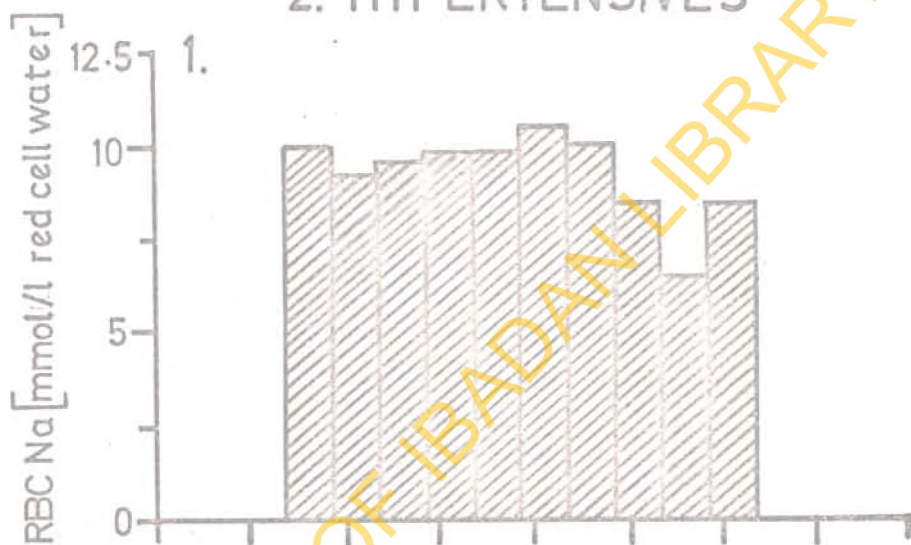
The higher content of sodium in red cells of hypertensive subjects is therefore a fundamental difference, not a difference introduced by differences



FIG. 5

Relationship between red cell sodium and age.

1. NORMOTENSIVES
2. HYPERTENSIVES



in RBC water content. Comparing the results for the normotensive Nigerian subjects with those of 7 caucasians whose results were presented, it was observed that the red cell sodium for the Nigerians was about 30% higher than those of the caucasians. On the other hand, the red cell potassium in the two groups were similar. (Table XXV).

8. Influence of Age (Fig 5 (I & II) Table V (1 & 2))

1. RBC Sodium

(a) Normotensives

In normotensive subjects, red cell sodium varied little with age until the age of 50 years. Above this age the red cell sodium fell so that at the age of 60 years, red cell sodium reached its minimum level of 7.6 mmol/l (Fig. 5, (1)).

(b) Hypertensives (Fig. 5 (2) Table V (2))

The graph shows 2 peaks with an anti-mode at age 50 years. There is an initial rise with age reaching a peak at the age of 32 years and falling to a minimum value at 50 years. There is a second rise which reaches a peak at age 65 before falling off. The 2 peaks are the same

TABLE V

EFFECT OF AGE ON RED CELL SODIUM AND POTASSIUM

1. NORMOTENSIVES

Age Range (YRS.)	RBC Na <sup>+</sup> mmo/1	RBC K <sup>+</sup> mmo/1	N as % of Total
14	-	-	-
19	9.9	88.3	10.9
24	9.5	99.2	34.8
29	9.6	87.9	24.0
34	9.8	87.5	13.0
39	9.8	86.7	8.6
44	10.5	84.4	4.3
49	10.0	84.9	2.8
54	8.2	80.8	1.3
59	7.6	78.0	0.1
64	8.5	85.6	0.2

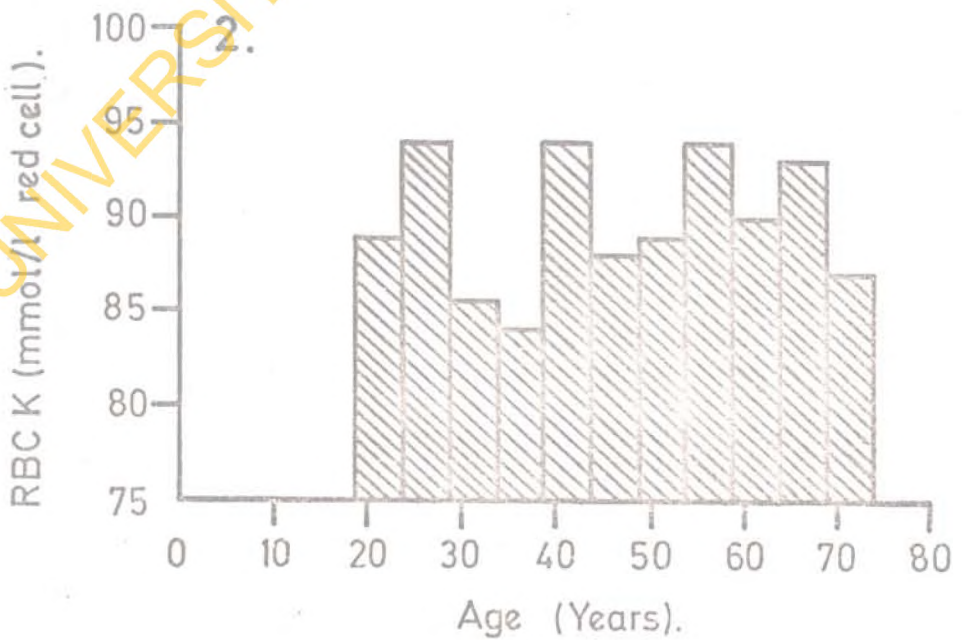
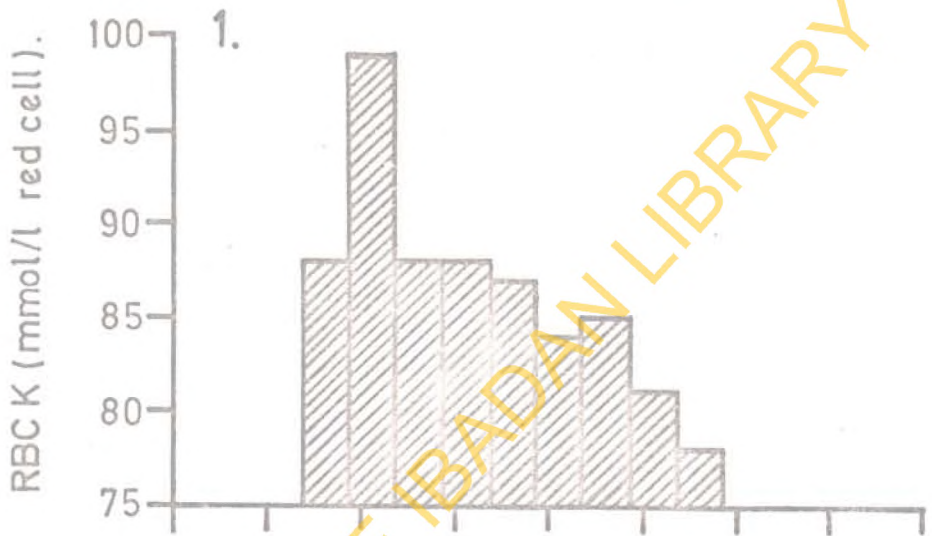
2. HYPERTENSIVES

Age Range (YRS.)	RBC Na <sup>+</sup> mmol/l	RBC K <sup>+</sup> mmol/l	N as % of Total
24	14.2	88.6	4.1
29	17.1	93.6	8.2
34	21.4	84.9	1.4
39	12.8	83.8	4.1
44	13.6	93.2	16.4
49	11.3	87.9	13.7
54	10.4	89.4	12.3
59	12.0	93.7	15.2
64	17.1	90.4	13.7
69	20.7	92.6	6.8
74	11.3	87.1	4.1

FIG.6

Relationship between red cell potassium and age.

- 1. NORMOTENSIVES.
- 2. HYPERTENSIVES.





value 21 mmol/l while the minimum value of 10.4 mmol/l recorded between the two modes is also similar to the value after 70.

## 2. RBC Potassium

### 1. Normotensives (Fig 6 (1))

Apart from a spike which occurs between ages 19 to 24, RBC potassium appears to be constant, and to vary very little until age 40, when a progressive fall with age sets in reaching a minimum of 78 mmol/l at age 60.

### 2. Hypertensives (Fig 6 (2) )

As in the case of RBC sodium, there is more than 1 peak in Figure 6 (2). There is an initial rise in red cell potassium, leading to a peak of 94 mmol/l at age 25 and falling to a minimum value of 84 mmol/l at about age 35. A second rise occurs with a peak at age 55 before a final falling off.

## 9. Genetic Factors (Table VI).

In Table VI, the results of analysis of the RBC sodium and potassium of three siblings and their mother was presented. Here again, wide inter-individual variations were observed in the erythrocyte

TABLE VI

ANALYSIS OF RESULTS OF 3 SIBLINGS AND THEIR MOTHER

Name	Sex	Status in the Family	Genotype	Age	RBC Sodium	RBC Potassium
M.G.	Female	Mother	AA	54	8.9	95.9
F.A.	Female	Sibling	AS	34	6.1	97.3
A.G.	Male	Sibling	AS	15	15.1	86.5
L.G.	Male	Sibling	AA	18	11.1	98.1

sodium content. Closer results were obtained for the erythrocyte potassium.

The wide inter-individual variations observed in siblings (who obviously have similar genetic inheritance) would suggest that environmental factors were probably more important determinants of RBC sodium content than genetic inheritance. It would be interesting to study the sodium content of monozygotic twins.

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10. Sex Differences. (Table VII (i) )

Normotensives:

Of a total of 908 normotensive control subjects, 870 were male and only 38 were female. The mean red cell sodium for the men was  $9.70 \pm 0.14$  mmol/l while that for the women was  $9.22 \pm 0.58$  mmol per litre. The mean red cell potassium for the men was  $90.3 \pm 0.32$  mmol/l while that for the women was  $90.3 \pm 1.59$  mmol/l.

Of the 71 normotensive subjects whose red cell water was determined, 48 were males, 13 were females, there was no record of the sex of the remaining 10 subjects.

The mean red cell water for the 48 male subjects was  $65.6 \pm 0.44\%$ . The mean red cell water for the 13 female subjects was  $67.0 \pm 0.78\%$ .

From the above results, there was no statistically significant sex differences in the RBC sodium, potassium and water content of the normotensive subjects studied.

Hypertensive Subjects (Table VII (ii) ).

AA Genotype

Red cell sodium, potassium and water were also



TABLE VII (i)

RBC SODIUM, POTASSIUM AND WATER CONTENT SEX DIFFERENCE

NORMOTENSIVES

Subjects	RBC Na <sup>+</sup> mmol/l	RBC K <sup>+</sup> mmol/l	Red Cell Water %
Normotensive Male	9.71 ± 0.14	90.3 ± 0.32	65.6 ± 0.44
N = 670	[2.4 - 28.27]	[68.1 - 117.27]	[56.4 - 68.7]
Normotensive Female	9.21 ± 0.58	90.3 ± 1.59	67.0 ± 0.78
N = 38	[5.0 - 20.97]	[68.9 - 114.97]	[60.8 - 71.77]
Difference between means	0.5 ± 0.59	0. ± 1.62	1.4 ± 0.90
P	> 0.01	N.S.	> 0.01

N.S. = Not significant

analysed to see if there are any sex differences in hypertensive subjects with the same genotype.

Twenty-two male subjects with genotype AA had a mean RBC Na of  $13.8 \pm 1.18$  mmol/l. The red cell potassium was  $94.5 \pm 2.90$  mmol/l and their red cell water was  $69.0 \pm 0.44$  mmol/l.

For the 22 women with genotype AA, the red cell sodium was  $13.2 \pm 1.30$  mmol/l, the red cell potassium was  $91.6 \pm 1.95$  mmol/l and the red cell water was  $67.9 \pm 0.84\%$ .

These results were summarised in Table VII (ii). There was no statistically significant difference in these parameters between the two sexes.

AS genotype (Tables XXXIII & XXXIV).

8 hypertensive male subjects with AS genotype had a mean RBC sodium of  $16.0 \pm 2.58$  mmol/l. Their red cell potassium was  $85.1 \pm 2.25$  mmol/l while their red cell water was  $68.0 \pm 0.74\%$ .

10 women with AS genotype also had a mean RBC sodium of  $15.6 \pm 1.94$  mmol/l. Their red cell potassium was  $97.3 \pm 3.97$  mmol/l, while their red cell water was  $67.9 \pm 1.21\%$ .

TABLE VII (ii)

SEX DIFFERENCES - HYPERTENSIVES

Subjects	RBC Na mmol/l	RBC K mmol/l	RBC Water %
Hypertensive Male	14.4 ± 0.01	91.4 ± 2.14	68.7 ± 0.38
N = 36	(5.2 - 28.0)	(73.3-118.3)	(65.4-72.6)
Hypertensive Female	13.9 ± 1.06	93.4 ± 1.85	67.9 ± 0.68
N = 32	(3.6 - 31.3)	(78.5-115.1)	(55.7 -72.6)
Difference	0.5 ± 1.06	2.0 ± 2.82	0.8 ± 3.70
P	> 0.5	> 0.1	> 0.1

There was no statistically significant difference in these parameters between male and female subjects.

Finally comparing the combined AA male and female with those for the AS male and female

it was observed that there is no statistically significant difference between the two sets of subjects.

As there was only one male subject with genotype AC, an analysis, such as was done for AA and AS genotypes above could not be done for genotype AC.

#### 10. Sex differences (Table VII (ii)).

The mean red cell sodium for 30 hypertensive male subjects was  $14.4 \pm 0.10$  mmol/l - the range was 5.2 to 28.0 mmol/l. Their mean red cell potassium was  $91.4 \pm 2.14$  mmol/l; the range was 73.3 to 118.3 mmol/l. Their red cell water was  $68.7 \pm 0.38\%$  with a range of 65.4 to 72.6%.

The mean red cell sodium for 32 hypertensive female subjects was  $13.9 \pm 1.06$  mmol per litre, with a range of 3.6 to 26.2 mmol/l. The mean red cell potassium was  $93.4 \pm 1.85$  mmol/l with a range of 78.5 to 115.1 mmol/l. The red cell water was  $67.9 \pm 0.68\%$  the range was 55.7 to 72.8%. There was no statistically significant difference in these 3 parameters between the male and female hypertensive subjects.



TABLE VIII

PLASMA SODIUM AND POTASSIUM IN NORMOTENSIVE  
CONTROLS AND HYPERTENSIVE SUBJECTS

Subjects	Na mmol/l	K mmol/l
All Controls	128.0 $\pm$ 0.50 (95.0-155.0; 209)	4.6 $\pm$ 0.08 (2.5-5.5; 200)
All Hypertensives	138.0 $\pm$ 0.64 (128.0-145.0; 39)	3.7 $\pm$ 0.08 (3.1-4.8; 36)
Difference	10.0 $\pm$ 0.81	0.9 $\pm$ 0.11
t =	12.35	8.18
p	< 0.001	< 0.001

In parentheses: range, number of subjects

Plasma Sodium (Table VIII, Fig. 7(A & B))

11. Normotensives and Hypertensives

Plasma sodium was determined in 209 normotensive controls. The mean value obtained was  $128 \pm 0.50$  mmol/l with a range of 95 to 155. In hypertensive subjects, plasma sodium was measured in 39 cases. The mean was  $138 \pm 0.64$  mmol/l and the range was 129 to 145.

The plasma sodium in hypertensives was higher than the plasma sodium in normotensives by 10 mmol/l. This difference is statistically significant.

12. Plasma Potassium (Table VIII, Fig. 8(A & B))

Plasma potassium was determined in only 200 of the 209 normotensive subjects whose plasma sodium was discussed above. Their plasma potassium was  $4.6 \pm 0.08$  mmol/l and the range was 2.5 to 5.5. Plasma potassium was determined in 35 of the 39 subjects above. The mean value was  $3.7 \pm 0.08$  mmol/l with a range of 3.1 to 4.8 mmol/l.

The plasma potassium in hypertensives was lower than that in normotensives by 0.8 mmol/l. This difference is statistically significant.

FIG. 7

The distribution of plasma sodium in normal and hypertensive Nigerians

A. NOMOTENSIVES

B. HYPERTENSIVES

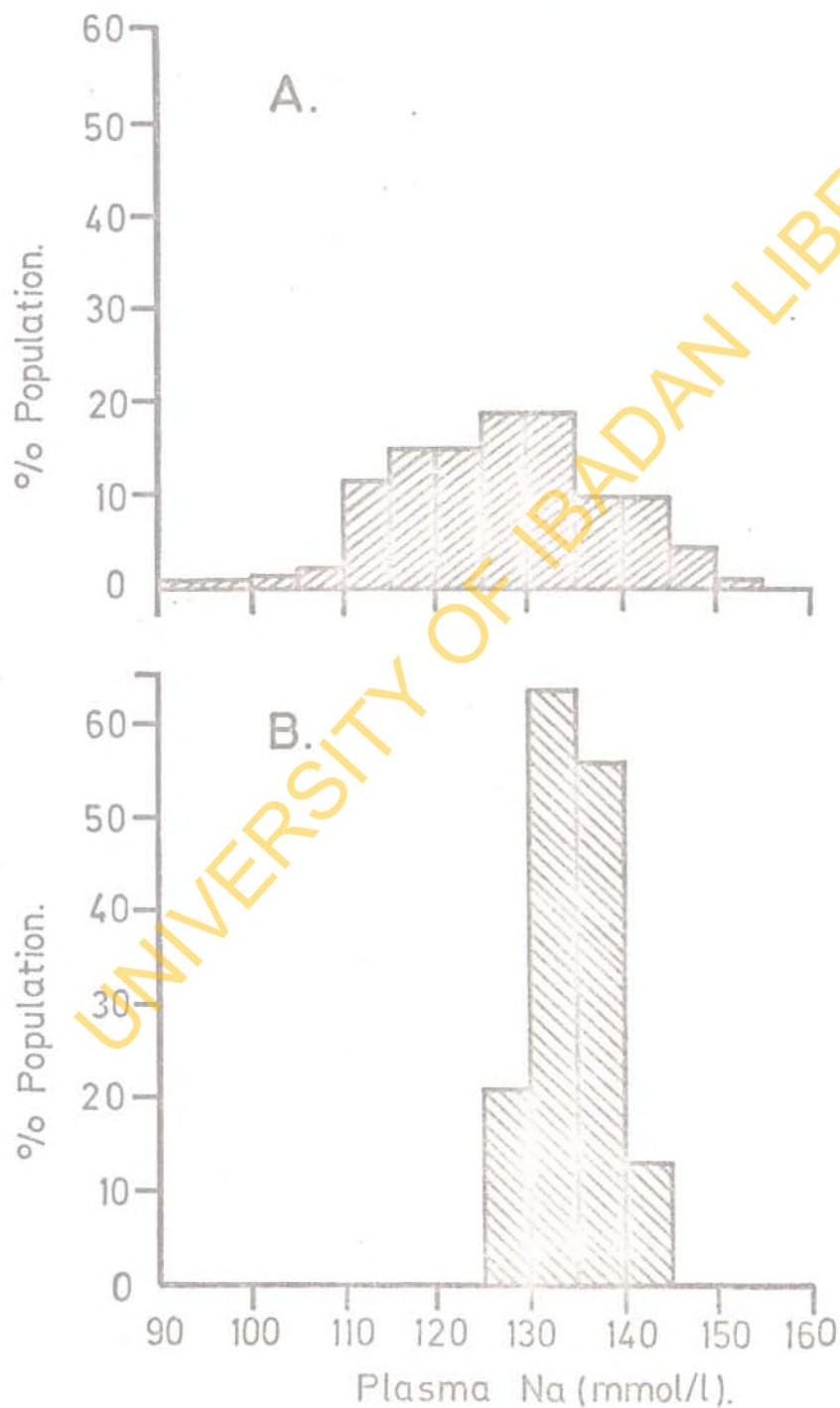
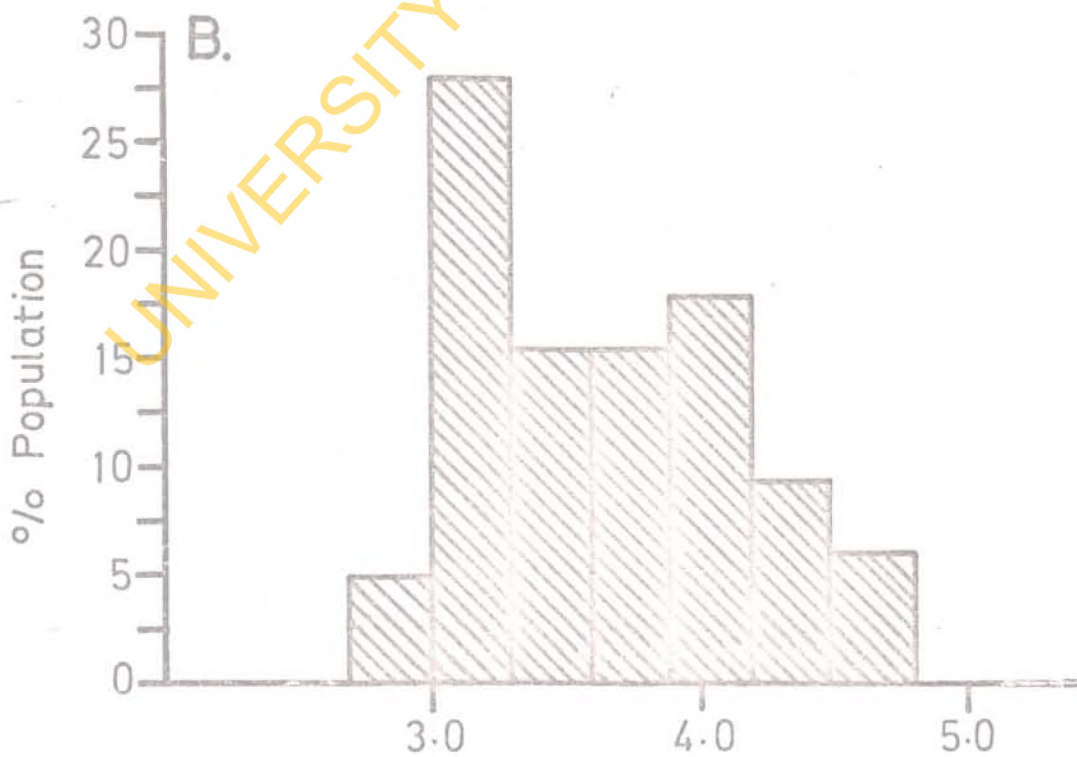
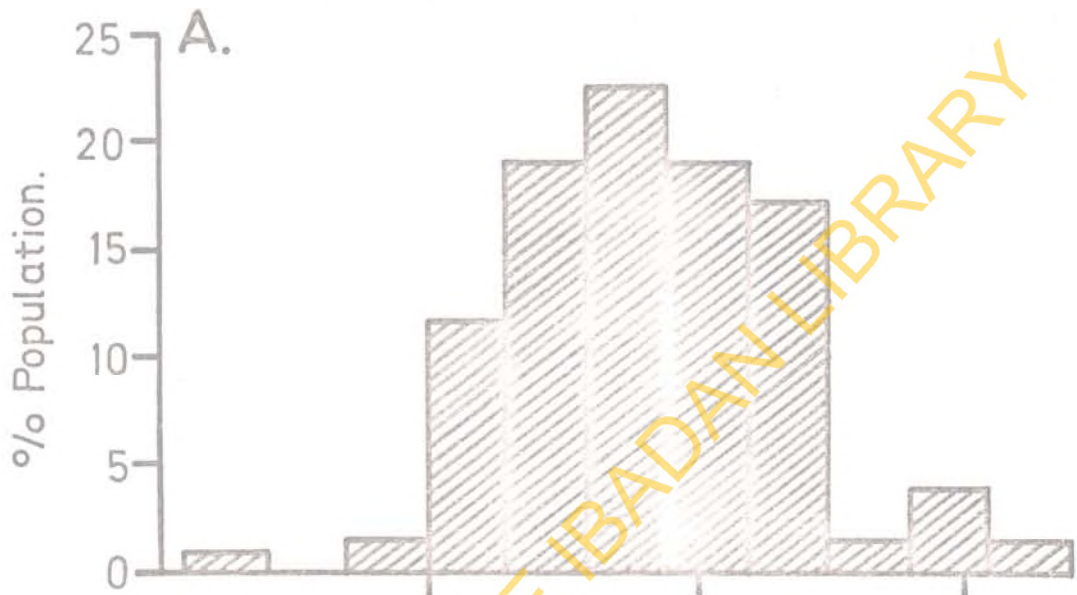


FIG. 8

The distribution of plasma potassium in normotensives and hypertensives.

A. NOMOTENSIVES.

B. HYPERTENSIVES.





Effect of treatment on RBC sodium and potassium  
Content in hypertensive subjects (Fig. 9. Table XVI & XVIII)

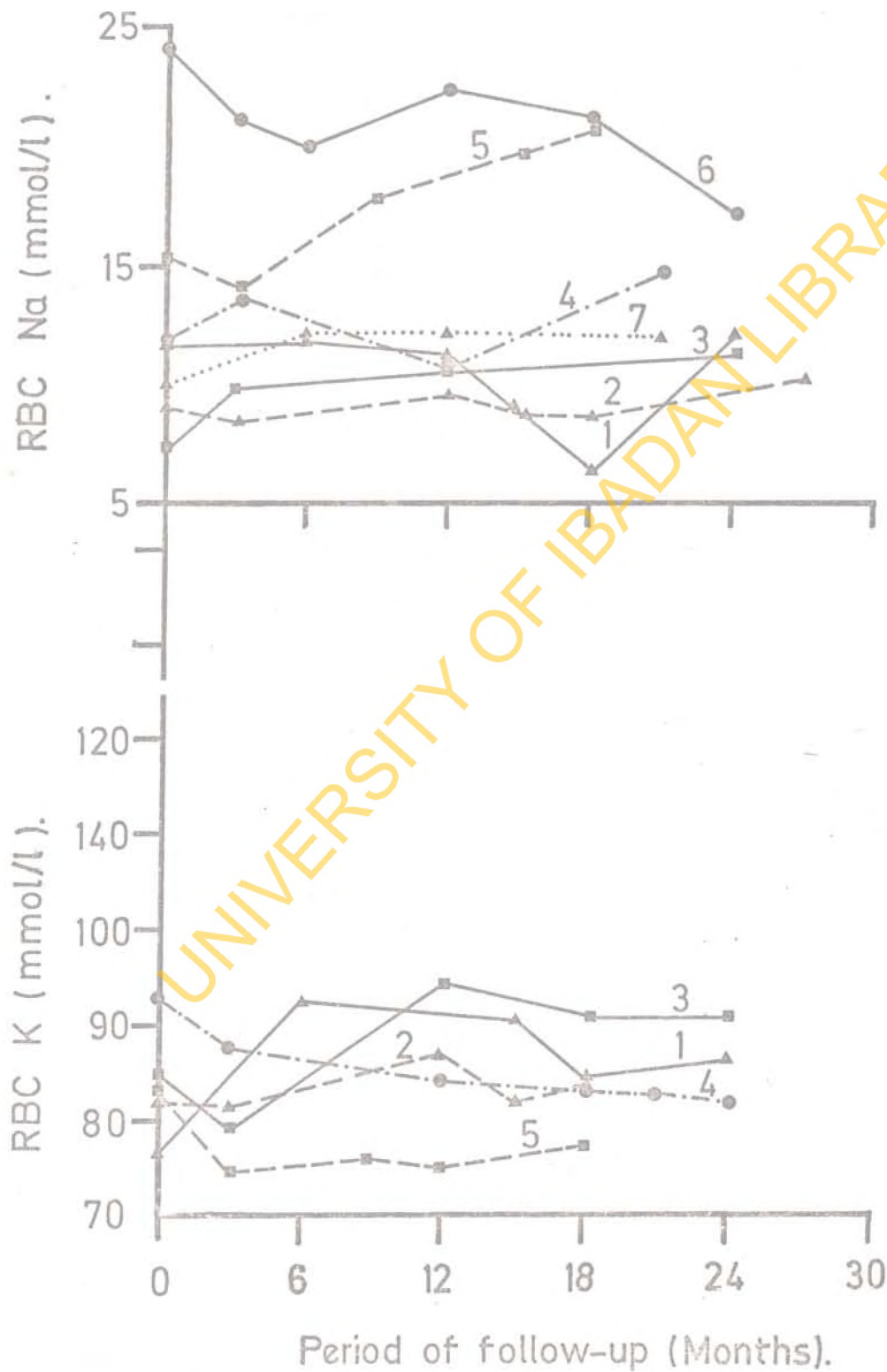
In fig. 9 the results of RBC sodium (7 subjects) and potassium (5 subjects) have been given over a period of 2½ years. The results at zero time represent the pre-treatment values, while the others are at 3 monthly intervals. These subjects are subjects 1 - 7 and 1 - 5 respectively in the appendix.

From these 2 graphs and from table XVIII. it was observed that RBC potassium remains unchanged during treatment. On the other hand RBC sodium is unpredictable, it may remain unchanged, it may decrease or increase, but mostly, it remains unchanged.

Fig 15 shows the graph of mean blood pressure against RBC Na (upper graph) and potassium (lower graph). There is no relationship between blood pressure and either sodium or potassium.

FIG. 9

Effect of treatment on RBC Na and K in Hypertensive patients.



CHAPTER 4

SODIUM AND POTASSIUM EFFLUX ACROSS THE

RED CELL MEMBRANE

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CHAPTER 4

Sodium and Potassium Efflux across the Red Cell Membrane

Outline

1. Passive Potassium Efflux
  - (a) brief description of procedure
  - (b) specimen calculation
  - (c) results
  - (d) within group comparisons
  
2. Sodium Efflux
  - (a) preparation of cells
  - (b) response to sodium load
  - (c) results of (2(c))
  - (d) active sodium efflux
    - (i) transport media
    - (ii) procedure
    - (iii) specimen calculation
  - (e) Results of 2(d)
  
3. Problems encountered during active sodium efflux studies.



### Passive Potassium Efflux

The method used to determine passive potassium efflux has been outlined in the chapter on methods. Further details of the procedure adopted are given below.

In the previous chapter sodium and potassium of erythrocytes was determined. It is however known that the intracellular cations are in a dynamic equilibrium with the extracellular cations, the concentration of ions in each compartment being a function of and also a determinant of the rate of movement of ions across the membrane separating the two compartments. In this chapter the rates of transport of the cations under study are determined with the same objective of characterising cation content and transport in red cells of normal and hypertensive Nigerians.

10 ml blood samples from the patients and controls were centrifuged to separate the red cells. The red cells so separated, were washed thrice with wash solution. Red cells obtained at the end of the third wash, were reconstituted with wash solution to a PCV of 40 - 50%. This value was decided upon

after preliminary experiments using 80% PCV as described by Kurantsin-Mills et al. (1974) gave inconveniently high K values in my experimental set-up using the EEL 150 flame photometer. A 40 - 50% PCV gave potassium concentrations that could be easily read on the flame photometer, and so this value was used. In addition, 40 - 50% haematocrit approximates better to the natural haematocrit than 80% does.

1 ml of the reconstituted RBC was then added to 9 ml of isotonic sucrose giving a 1 in 10 dilution for the reconstituted cells and a final PCV of  $\frac{1}{10} \times X\%$  where X is the PCV of the reconstituted cells.

For each subject, 20 tubes containing 10 ml of these diluted red cells were set up, and incubated at 25°C for periods of up to 4 hours. The tubes were divided into 2 groups. In the first, two tubes were removed from the water bath at intervals of 10 minutes up to 1 hour. In the second group, two tubes were again removed at 1, 2, 3 and 4 hours. In this way, blood samples were obtained after incubation at hourly intervals up to 4 hours.

The content of each tube was then prepared for the analysis of its potassium concentration as follows:

The sample was centrifuged and the supernatant carefully pipetted out. 1 ml of the supernatant was then diluted 1 in 5 and the potassium concentration read in the EEL 150 flame photometer as described in detail under "Methods" (Chapter II).

#### Specimen Calculation

Patient O.B. (hypertensive subject No. 8 Table XI) had an artificial PCV of 42%. 1 ml of her washed cells of PCV 42% was added to 9 ml of isotonic sucrose.

The actual PCV of RBC used for K permeability studies

$$= \frac{1}{10} \times X$$

$$\text{Actual PCV} = \frac{42}{10} = 4.2\%$$

The 10 ml reconstituted samples of RBC (PCV 4.2%) was incubated for 10 minutes. At the end of the incubation period, a 1:5 dilution of the supernatant gave a flame photometer reading of 35 mmol/l.

$$\begin{aligned} \text{Actual flame photometer reading undiluted samples} \\ = 35 \times 5 \text{ mmol/l} \end{aligned}$$

TABLE IX

PASSIVE K<sup>+</sup> EFFLUX RATE PER MINUTE IN NORMOTENSIVES (mmol/l)(a) INDIVIDUAL RESULTS

Genotype	10th Min.	20th Min.	30th Min.	40th Min.	50th Min.	60th Min.
AA	2.7	1.3	0.8	0.6	0.5	0.4
AA	2.6	1.5	0.9	0.7	0.4	0.4
AA	2.8	1.7	0.8	0.6	0.5	0.4
AS	2.5	1.2	0.7	0.7	0.6	0.5
AS	2.9	2.0	1.5	0.8	0.5	0.5
AS	2.4	1.8	1.3	0.9	0.8	0.6
AS	2.5	1.3	0.8	0.7	0.6	0.5
AC	2.3	1.0	0.7	0.6	0.5	0.5
AC	1.9	0.9	0.8	0.7	0.6	0.6
Mean +S.E.M.	2.5±0.10	1.4±0.12	0.9±0.09	0.7±0.03	0.5±0.04	0.5±0.03

(b) MEAN K EFFLUX ACCORDING TO GENOTYPES (mmol/l)

	10th Min.	20th Min.	30th Min.	40th Min.	50th Min.	60th Min.
1	2.7 ± 0.06	1.5 ± 0.12	0.8 ± 0.03	0.6 ± 0.03	0.5 ± 0.03	0.4 ± 0
2	2.6 ± 0.11	1.6 ± 0.19	1.1 ± 0.19	0.8 ± 0.05	0.6 ± 0.06	0.5 ± 0.03
3	2.1 ± 0.20	1.0 ± 0.05	0.8 ± 0.05	0.7 ± 0.05	0.6 ± 0.04	0.6 ± 0.04

1 = AA(3 subjects)  
 2 = AS(4 subjects)  
 3 = AC(2 subjects)



TABLE X

## PASSIVE K EFFLUX RATE PER HOUR (mmol/Hr.) IN NORMOTENSIVES

(a) INDIVIDUAL RESULTS

Genotype	1st Hour	2nd Hour	3rd Hour	4th Hour
AA	0.63	0.31	0.26	0.21
AA	0.63	0.37	0.29	0.19
AA	0.50	0.35	0.27	0.21
AS	0.73	0.52	0.33	0.23
AS	0.39	0.26	0.22	0.19
AS	0.40	0.18	0.17	0.15
AS	0.20	0.18	0.17	0.15
AC	0.47	0.25	0.24	0.23
AC	0.31	0.15	0.12	0.11
Mean <sup>†</sup>	0.47	0.29	0.23	0.19
S.E.M.	+0.06	+0.04	+0.02	+0.01

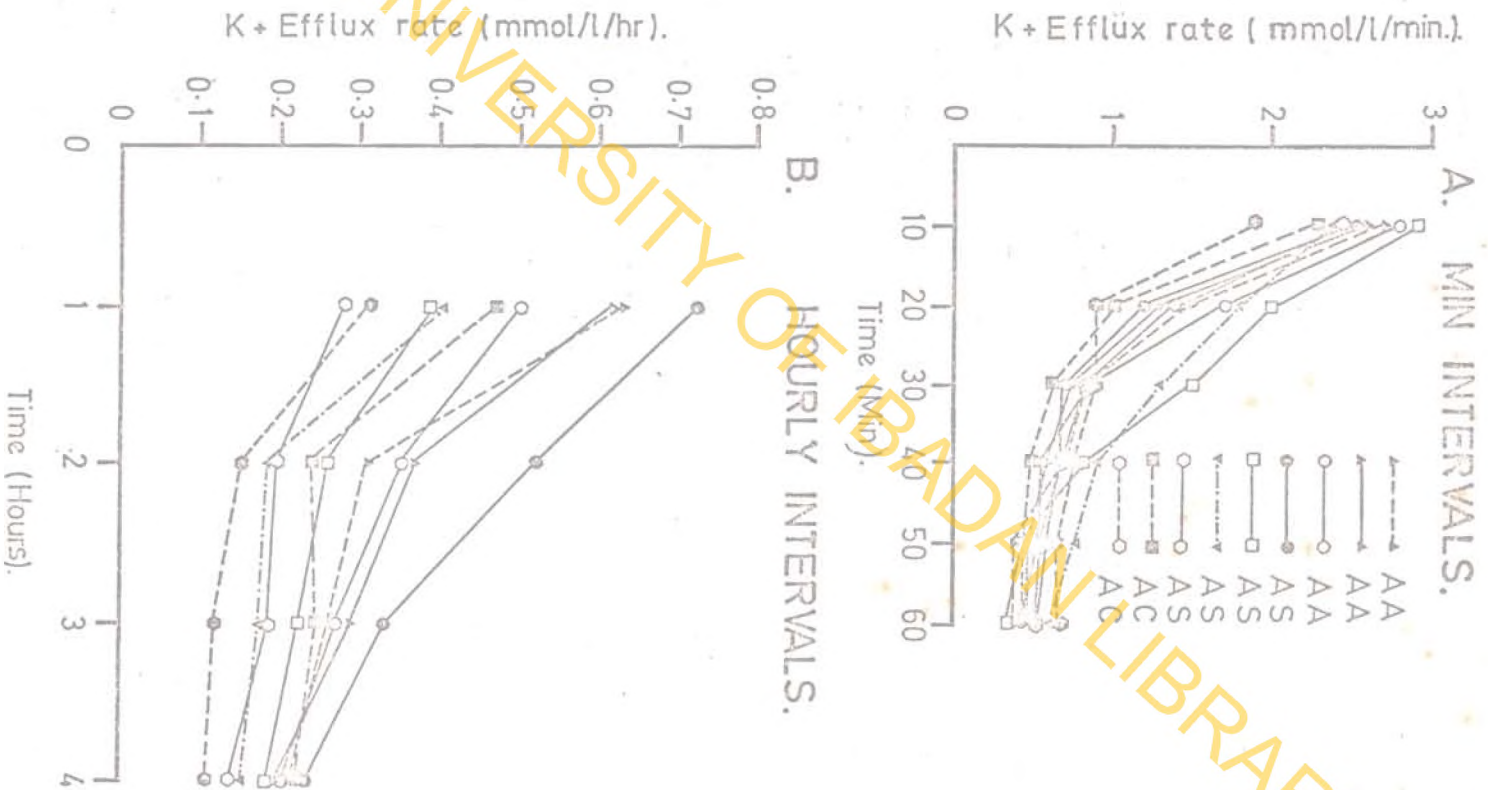
(b) MEAN K EFFLUX ACCORDING TO GENOTYPES (mmol/Hr.).

Subjects	Mean K Efflux Per Hour			
	1st Hour	2nd Hour	3rd Hour	4th Hour
AA (3)	0.59±0.04	0.34±0.02	0.27±0.01	0.20±0.01
AS (4)	0.45±0.11	0.28±0.09	0.19±0.02	0.22±0.05
AC (2)	0.39±0.07	0.20±0.04	0.19±0.06	0.17±0.07

In parentheses: number of subjects  
 S.E.M. = Standard Error of Mean.

FIG.10

Normotensive passive potassium efflux rate (mmol/l/min.) in 9 subjects.



Total K flux into the isotonic sucrose medium in 10 minutes =  $35 \times 5$  = flame photometer dilution factor of 200.

For RBC of 100% PCV, passive flux in 10 minutes.

$$= 35 \times 5 \times \frac{1}{200} \times \frac{100}{42}$$

Passive K flux per minute in the first 10 minutes

$$= 35 \times 5 \times \frac{1}{200} \times \frac{100}{4.2} \times \frac{1}{10}$$
$$= \frac{175}{84} = 2.08 \text{ mmol/l}$$

Results (Tables IX, X, XI and XII; Figures 10a and b, 11a and b).

Results of passive potassium efflux in 8 hypertensive subjects and 9 controls are presented in the above Tables and Figures. The results were obtained from 3 different sets of experiments. In the first set, the first three hypertensives and the first three controls were involved. In the second set of experiments, the next three hypertensives and the next 3 controls were involved and in the third the last 2 hypertensives and the last 3 controls were involved.

Both Figures X (a and b) and XI (a and b) showed exponential curves. The rate of passive

TABLE XI

HYPERTENSIVE PASSIVE POTASSIUM EFFLUX RATES -

INDIVIDUAL RESULTS (mmol/l/min.)

1. EFFLUX RATE AT MINUTE INTERVALS

Genotype	10th Min.	20th Min.	30th Min.	40th Min.	50th Min.	60th Min.
AA	0.41	0.19	0.13	0.07	0.09	0.08
AA	0.38	0.24	0.16	0.11	0.11	0.10
AS	0.43	0.19	0.16	0.13	0.13	0.09
AA	0.20	0.10	0.05	0.04	0.04	0.04
AA	0.26	0.11	0.09	0.05	0.04	0.04
AA	0.47	0.19	0.11	0.12	0.10	0.09
AA	0.33	0.19	0.12	0.11	0.11	0.09
AA	0.21	0.15	0.07	0.05	0.04	0.04
Mean						
+ S.E.M.	0.32±0.04	0.18±0.01	0.10±0.01	0.08±0.01	0.07±0.01	0.07±0.01

2. EFFLUX RATE AT HOURLY INTERVALS

Subject	Genotype	1st Hour	2nd Hour	3rd Hour	4th Hour
1	AA	0.08	0.06	0.03	0.03
2	AA	0.12	0.09	0.04	0.04
3	AS	0.11	0.07	0.06	0.05
4	AA	0.04	0.03	0.03	0.02
5	AA	0.09	0.04	0.02	0.02
6	AA	0.13	0.05	0.04	0.03
7	AA	0.10	0.04	0.04	0.03
8	AA	0.04	0.02	0.01	0.01
Mean					
+ S.E.M.		0.09±0.01	0.05±0.01	0.04±0.01	0.03±0.01

S.E.M. = Standard Error of Mean.



TABLE XII

PASSIVE K EFFLUX RATES IN HYPERTENSIVES (mmol/l)

ANALYSIS ACCORDING TO GENOTYPES

1. MINUTE INTERVALS (mmol/l/min.)

	10th Min.	20th Min.	30th Min.	40th Min.	50th Min.	60th Min.
1	0.32 $\pm$ 0.04	0.18 $\pm$ 0.01	0.10 $\pm$ 0.01	0.08 $\pm$ 0.01	0.07 $\pm$ 0.01	0.07 $\pm$ 0.01
2	0.43	0.19	0.16	0.13	0.13	0.09

1 = AA (7 subjects)

2 = AS (1 subjects)

2. HOURLY INTERVALS (mmol/l/hr.)

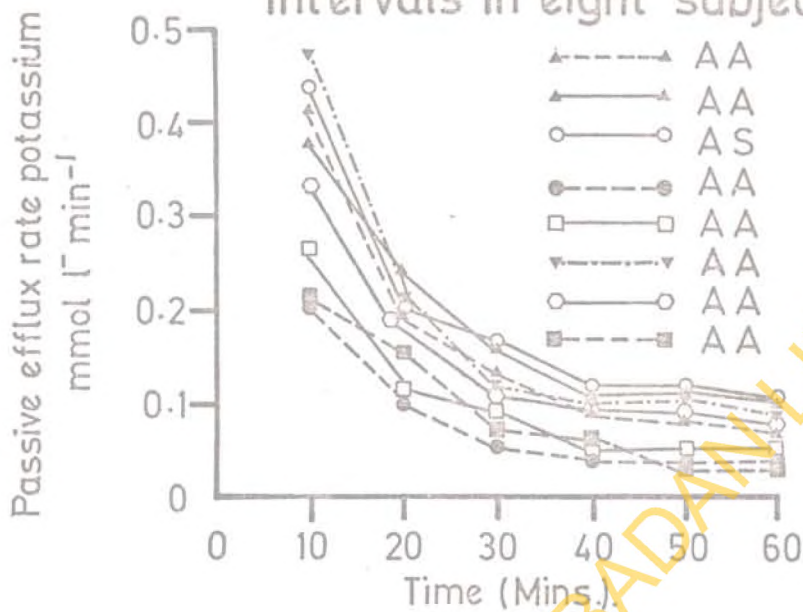
Subject	1st Hour	2nd Hour	3rd Hour	4th Hour
AA (7 subjects)	0.09 $\pm$ 0.01	0.05 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01
AS (1 subject)	0.11	0.07	0.06	0.05

In parentheses: number of subjects.

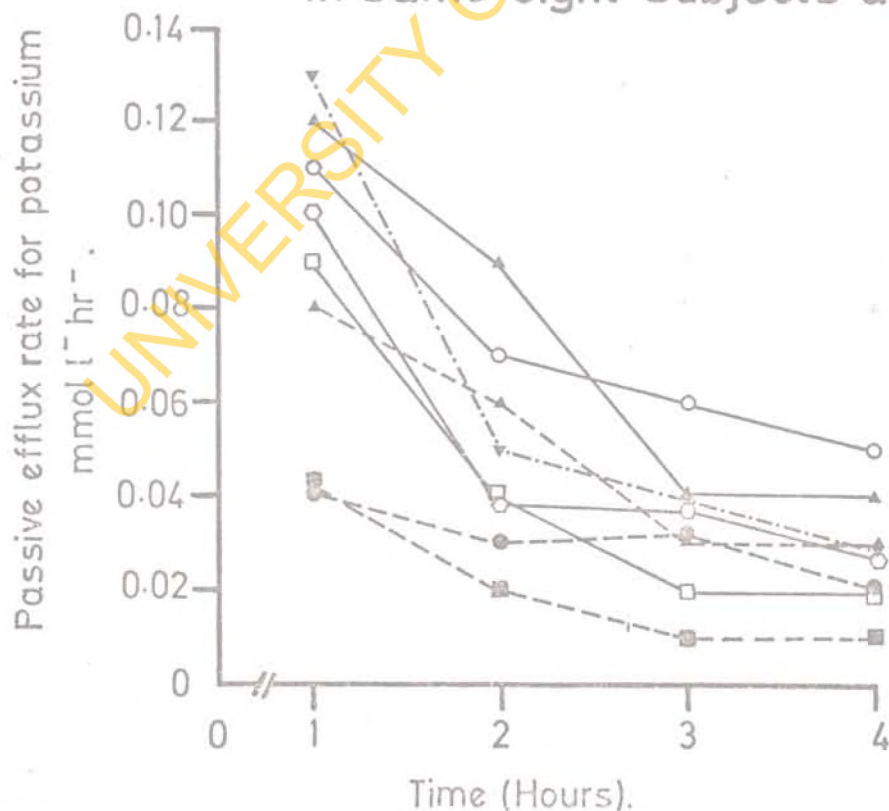
FIG. 11

Hypertensive passive potassium efflux rate (mmol/l min.).

A. Efflux rate at ten minute intervals in eight subjects.



B. Efflux rate at hourly intervals in same eight subjects as above.



potassium efflux was fastest at 10 minutes in both the hypertensives and controls, but more so in the controls. This rate slowed considerably between the 20th and 40th minute and the curve was almost flat thereafter.

After the first hour the rate of passive efflux is considerably reduced. At the end of the third hour, the curve is flat especially in the Hypertensives. Efflux rate during the first 10 minutes in controls was  $2.5 \pm 0.04$  mmol/l/min but was  $0.5 \pm 0.03$  mmol/l/min during the last 10 minutes of the first hour. In hypertensives, efflux rate per minute was  $0.34 \pm 0.04$  mmol/l/min, and  $0.08 \pm 0.01$  mmol/l/hr in the first and last 10 minutes of the first hour respectively. The difference between the two groups of subjects was statistically significant ( $P < 0.001$ ) at all the intervals measured.

#### Within -group Comparisons

Table XII shows that within the normotensive group, efflux rates of the erythrocytes with Hb genotypes AA, AS and AC are closely similar. Again, looking at Fig. 10 it is observed that individual plots of the efflux rates for Hb, AA AS and AC in the

TABLE XIII

COMPARISON OF PASSIVE POTASSIUM EFFLUX RATES  
IN NORMOTENSIVES AND HYPERTENSIVES

1. EFFLUX RATE AT THE MINUTE INTERVALS

(Taken as the rate in the 1st. 10 minutes)

Subjects	Mean and Range	Difference
Normotensives (9)	$2.5 \pm 0.10$ $[1.9 - 2.7]$	$2.18 \pm 0.10$
Hypertensives (8)	$0.32 \pm 0.04$ $[0.2 - 0.4]$	t = 21.8 p < 0.001

2. EFFLUX RATE AT THE HOURLY INTERVALS

(Taken as the rate in the 1st. 1 hour)

Subjects	Mean and Range	Difference
Normotensives (9)	$0.47 \pm 0.06$ $[0.28 - 0.73]$	$0.38 \pm 0.06$
Hypertensives (8)	$0.09 \pm 0.01$ $[0.04 - 0.13]$	t = 6.33 p < 0.001



normotensive subjects overlap. There is no statistically significant difference in  $K^+$  efflux rates of the 3 groups.

In the hypertensive group, of the 8 subjects whose results are presented, only 1 subject was of AS genotype, the other seven were AA. The same kind of intra-group comparison as above could not be carried out but the plot of individual values for each subject showed the values for the AS subject within the limits covered by the AA subjects.

### Sodium Efflux

#### (a) Preparation of Cells

The procedure used in studying sodium transport across the erythrocyte membrane had been outlined in the chapter on methods.

Further details are given below:

10 ml samples of blood were obtained from hypertensive subjects and controls. The samples were spun and the plasma and topmost layer of cells were aspirated. 4 ml of packed cells were then pipeted into fresh plastic bottles. To these packed cell samples, 3 ml of loading medium (also known as cold storage medium) was added. The cold

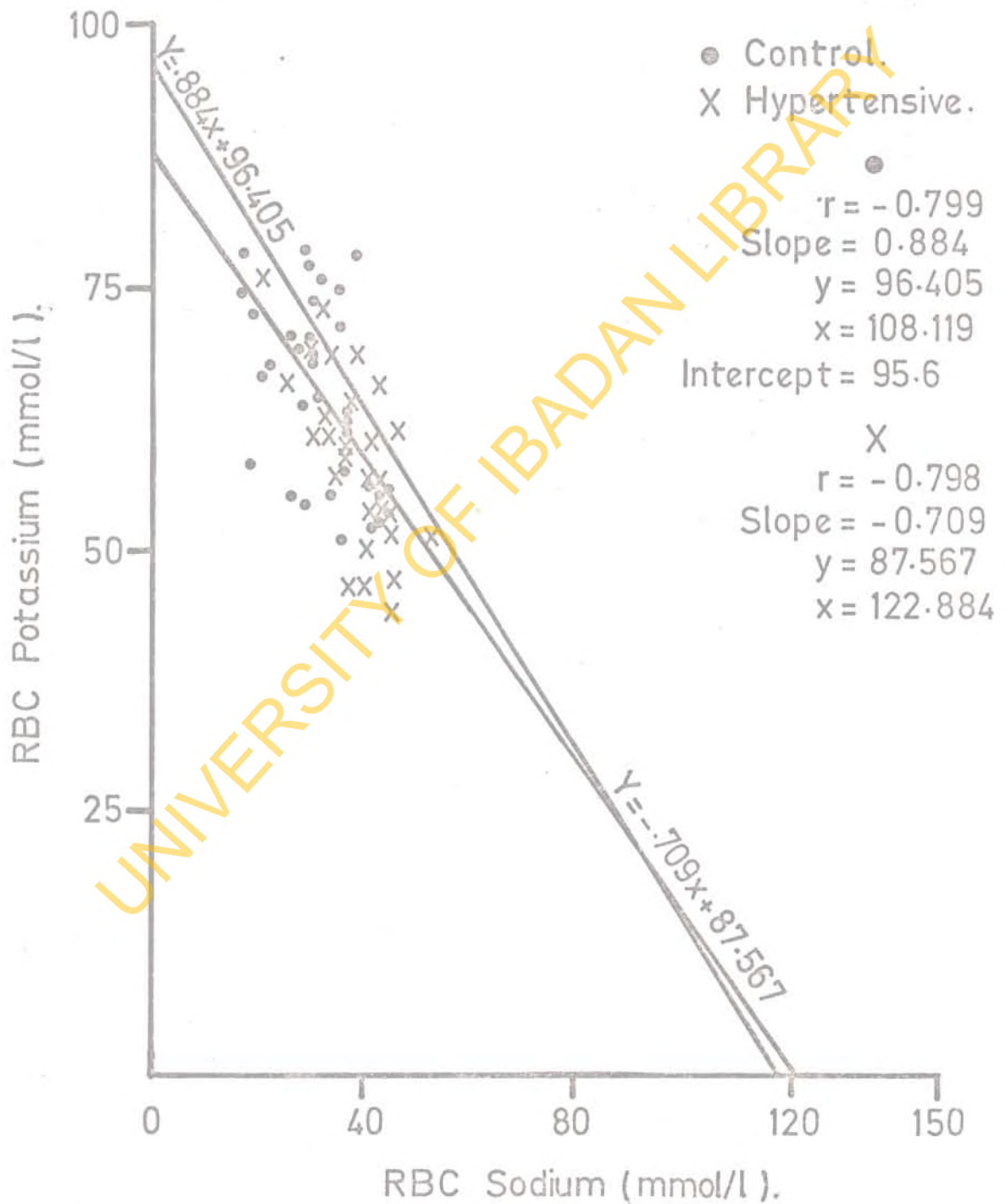
storage medium of Post and Jolly (1967), is a high sodium medium consisting of NaCl, Na<sub>2</sub>HPO<sub>4</sub>, HCl, MgCl<sub>2</sub>, adenosine, glucose and albumin in proportions already outlined under methods.

The cells were well mixed with the loading (Cold storage) medium and stored at 4°C for 10 days. The medium tended to become increasingly acid during cold storage at 4°C. Since an acid medium would lead to haemolysis, the cold storage medium was changed every 48 hours, consequently the pH of the medium was maintained as close as possible to  $7.4 \pm 0.2$ . The medium was never kept for more than 96 hours, any left over, after changing the red cell media, was stored frozen (-4°C).

At the end of this storage period, the cells were spun, the supernatant was aspirated, and the cells were washed three times, with 5 ml volumes of cold (4°C) wash solution. After each wash, the cells were spun, and the supernatant was aspirated. At the end of the third wash, the supernatant contained less than 10 mmol/l of sodium. The RBC was then reconstituted with the wash solution to a PCV of about 50%.

FIG. 12

Response of RBC potassium to sodium loading.



(b) Response to Sodium Load

0.1 ml aliquots of Na-loaded reconstituted RBC obtained above were taken into 9.9 ml deionised water to form a 1:100 dilution; the rest of the reconstituted cells were used for sodium transport experiments. Red cell sodium and potassium were estimated on the lysed cells, as described for RBC sodium and potassium content.

(c) Results

[Fig 12]

In both hypertensive and controls, the RBC membrane responds to a sodium load by passively increasing its intracellular sodium and reducing its intracellular potassium (Table XIVa). The initial RBC sodium in hypertensives was higher than the initial value in normotensives confirming data obtained in Chapter 3.

The increase in intracellular sodium is much higher for hypertensives than controls. The difference between the two groups is statistically significant. The fall in intracellular potassium is also greater in hypertensives than in normotensives. The difference here again is statistically significant (Table XIVb).



TABLE XIV

## RESPONSE OF RBC MEMBRANE TO PASSIVE SODIUM LOADING

(a) Subjects	Initial RBC Na <sup>+</sup> mmol/l	Final RBC Na <sup>+</sup> mmol/l	Initial RBC K <sup>+</sup> mmol/l	Final RBC K <sup>+</sup> mmol/l
Controls (40)	9.9 ± 0.53	29.4 ± 1.22	86.0 ± 1.30	66.0 ± 1.01
Hypertensives (29)	13.8 ± 0.89	38.7 ± 1.41	91.2 ± 0.94	56.3 ± 2.25

(b) Subjects	Gain in RBC Na <sup>+</sup> during sodium loading (mmol/l)	Loss in RBC K <sup>+</sup> during sodium loading (mmol/l)
Controls	19.5 ± 1.33	20.0 ± 1.65
Hypertensives	24.9 ± 1.67	34.9 ± 2.44
Difference	5.4 ± 2.13	14.9 ± 2.95
t	2.54	5.05
p	0.01	0.001

(d) Active Sodium Efflux

Red blood cells loaded with sodium as described under 'preparation of Cells' and washed and reconstituted to a PCV of about 50% were used for sodium transport. These samples were the same samples from which aliquots were taken for determination of 'response to sodium load'. Experiments were set up in duplicate for active and passive transport. For sodium transport experiments, 4 test-tubes were set up for each sample: 2 for net transport (tubes 1 and 2), 2 for passive transport (tubes 3 and 4). The experiments were set up such that both the hypertensive samples and control samples were done simultaneously. This was to avoid discrepancies in results brought about by fluctuations in electricity supply or any other local fault.

(i) Transport Media

Medium A (For Net Transport)		Medium B (For Passive Transport)
KCl	110 mmol/l	110 mmol/l
Na <sub>2</sub> HPO <sub>4</sub>	25 mmol/l	25 mmol/l
HCl	2 mmol/l	2 mmol/l
MgCl <sub>2</sub>	2 mmol/l	2 mmol/l

Glucose	10 mmol/l	10 mmol/l
Adenosine	3 mmol/l	3 mmol/l
Albumin	1 g/l	1 g/l
Ouabain	Nil	0.1 mmol/l

(ii) Procedure

The above 2 media were used for suspending the washed cells for the transport studies. Medium A was used for net transport and medium B for passive transport.

Into each of tubes 1 and 2, 4.8 ml of medium A was put, while into each of tubes 3 and 4, 4.8 ml of medium B was put. 0.2 ml aliquots of the washed loaded red cells were then added to each of the four tubes. The tubes were well shaken and placed in a water bath at 37°C for four hours. During incubation, the cells were shaken by hand from time to time, and the movements of a mechanical stirrer in the water bath also kept the cells well mixed with the incubation medium.

After incubation, the cell suspension was centrifuged and the cells were separated from the media. The cells were washed immediately

with 10 ml cold (4°C) wash solution, and centrifuged. Sodium efflux was then estimated in the cells.

Sodium efflux was not estimated in the media because the medium contained so much sodium (25 mmol/l)  $\text{Na}_2\text{HPO}_3$  that the small amount of sodium efflux was completely eclipsed. Consequently, net sodium efflux did not have an appreciably different value from passive efflux.

(iii) Specimen calculation

Active sodium efflux was estimated in the red cells by taking the difference in flame photometer reading between the red cell sodium of tubes 1 and 2 (net transport) and 3 and 4 (passive transport).



Active sodium efflux in 4 hours.

$$= r \times d \times \frac{100}{h} \times \frac{1}{200}$$

where r = flame photometer reading

d = dilution factor of 100

h = PCV of transport cells

200 = flame photometer dilution factor

Active sodium transport in 4 hours

$$= r \times 100 \times \frac{100}{h} \times \frac{1}{200}$$

$$= r \times \frac{50}{h} \text{ mmol/l/hr}$$

Active sodium transport in 1 hour

$$= r \times \frac{50}{4h} \text{ mmol/l/hr.}$$

(iv) Results (Table XV; Figs 12, 13 and 14).

TABLE XV

## ACTIVE SODIUM TRANSPORT - INITIAL RESULTS

	Red Cell Sodium (mmol/l)		Efflux Rate Constant (per hour)	
	Patient	Control	Patient	Control
12/7/76-22/7/76				
1.	47.9	39.5	0.065	0.266
2.	64.7	23.0	0.042	0.157
3.	-	46.8	--	0.108
4.	-	32.9	--	0.199
9/2/77-19/2/77				
5.	43.0	24.4	0.126	0.303
6.	38.7	24.0	0.150	0.163
7.	55.6	27.7	0.077	0.242
8.	30.5	-	0.184	--
9.	53.0	-	0.089	--
22/2/77-4/2/77				
10.	39.7	17.4	0.141	0.224
11.	42.7	28.9	0.080	0.156
12.	31.4	45.8	0.124	0.142
13.	25.6	29.7	0.164	0.229
14.	44.1	26.0	0.136	0.223
15.	33.7	41.0	0.142	0.129
22/3/77-1/4/77				
16.	36.6	-	0.074	--
17.	41.5	-	0.084	--
18.	36.8	27.1	0.052	0.103

Mean plus Std. Error of Mean (S.E.M.)

41.6 $\pm$ 2.51    31.01 $\pm$ 2.39    0.11 $\pm$ 0.01    0.19 $\pm$ 0.02

Dates indicate duration of RBC cold storage.

TABLE XVI

ACTIVE SODIUM TRANSPORT - INITIAL RESULTS

TOTAL ACTIVE EFFLUX (mmol/l/hr.)

(Same Cells as for A)

	In 4 hours		In 1 hour	
	Patient	Control	Patient	Control
1.	12.3	42.1	3.1	10.5
2.	10.7	14.2	3.7	3.5
3.	-	20.1	-	5.0
4.	-	25.3	-	6.6
5.	21.5	23.4	5.4	7.4
6.	23.0	15.5	5.8	3.9
7.	17.0	26.6	4.3	6.7
8.	22.5	--	5.6	-
9.	18.9	--	4.7	-
10.	22.5	15.7	5.6	3.9
11.	13.5	17.8	3.4	4.5
12.	15.7	26.0	3.3	6.5
13.	15.7	27.0	4.2	6.8
14.	23.9	23.1	6.0	5.8
15.	19.2	21.2	4.8	5.3
16.	10.9	--	2.7	-
17.	14.1	--	3.5	-
18.	7.1	11.2	1.9	2.8

Mean plus S.E.M.

16.9±1.27

22.6±2.46

4.23±0.32

5.7±0.53

### Active Sodium Efflux

Pre Treatment Results (Figs. 13 & 14, Tables XV & XVI).

Active sodium efflux rate for the 14 controls was  $5.70 \pm 0.53$  mmol/l/hr, while that for subjects 1 - 16 of the newly diagnosed hypertensives (see appendix) was  $4.23 \pm 0.32$  mmol/l/hr. The normotensive sodium efflux rate was higher than that for the hypertensives by 1.47 mmol/l, but the difference was not statistically significant.

The efflux rate constant was higher in normotensives than in hypertensives by 0.08/hr. This difference was statistically significant.

Figure 13 shows the graphs for rate constant against red cell sodium in controls and hypertensives. There is a negative correlation between the red cell sodium and active efflux rate constant. The correlation is better in hypertensives ( $r = -0.53$ ) than in controls ( $r = -0.43$ ). The difference between the two is statistically significant (Table XVII).

In figure 14 the active sodium efflux has been plotted against the red cell sodium. In controls, the relationship between the 2 is linear and is



FIG.13

The relationship between the red cell sodium concentration and the rate constant for active sodium efflux from the cells in 16 hypertensive subjects and 14 controls.

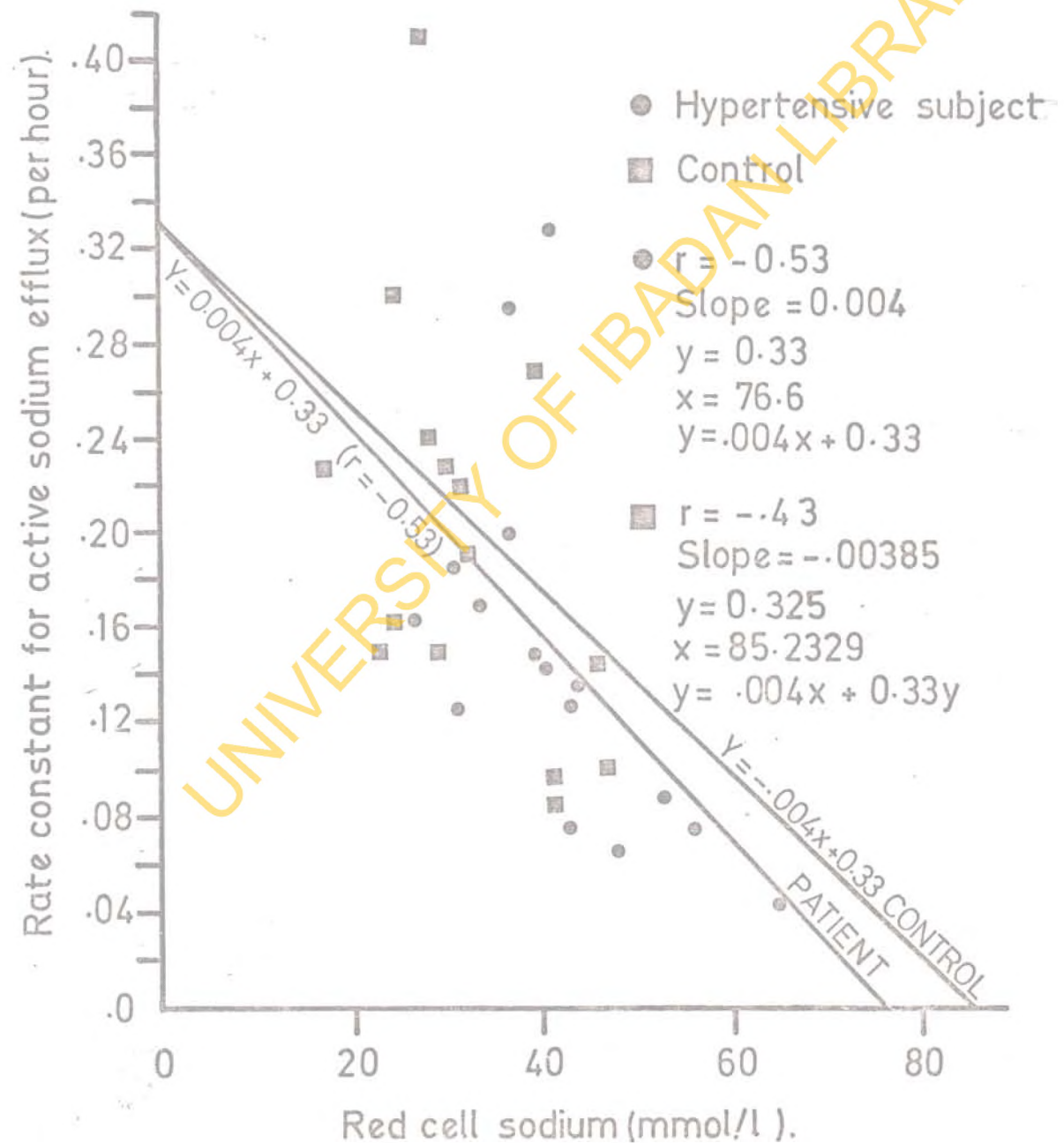
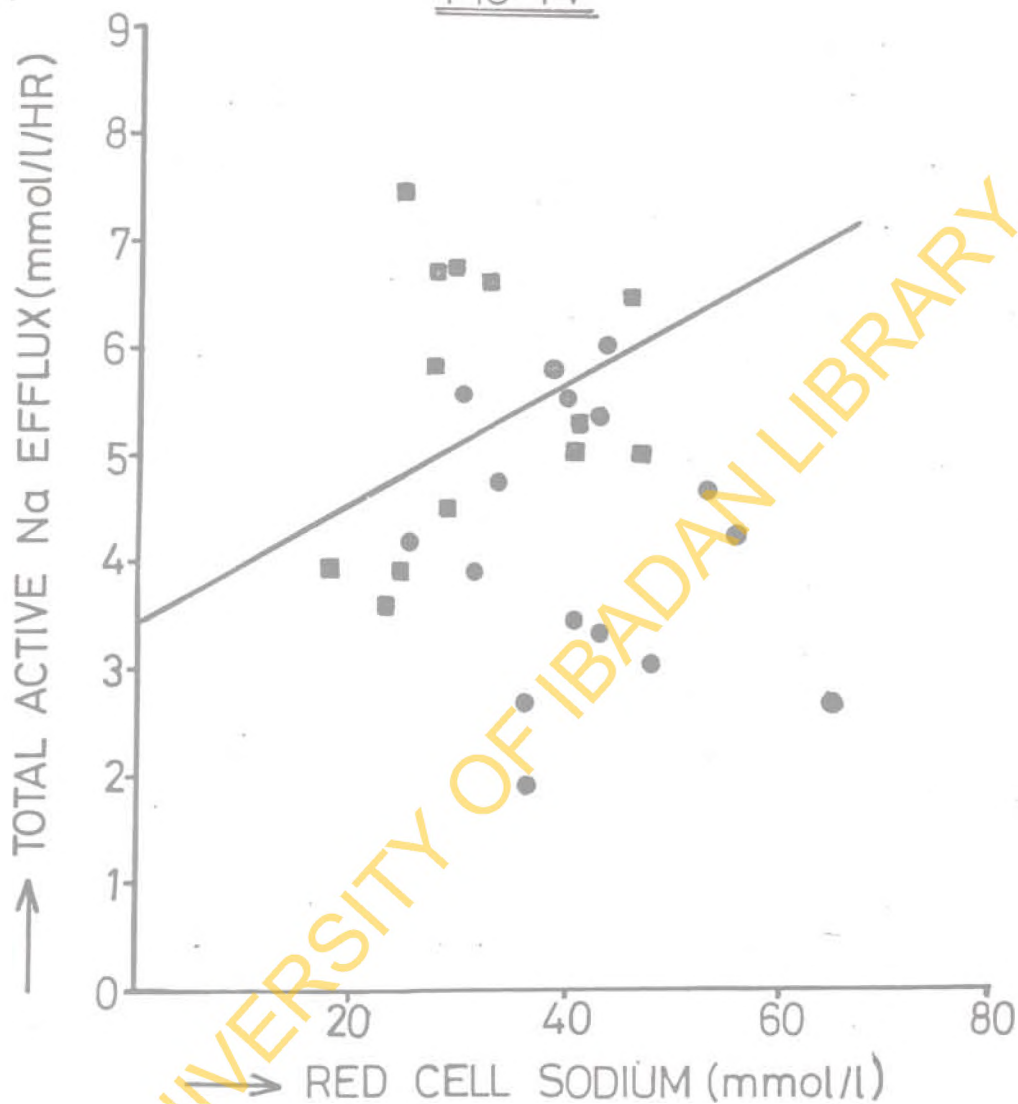


FIG. 14



THE RELATIONSHIP BETWEEN RED CELL SODIUM CONCENTRATION AND THE TOTAL AMOUNT OF SODIUM ACTIVELY PUMPED FROM THE CELLS: 16 HYPERTENSIVE SUBJECTS (●) AND 14 CONTROLS (■).

■ : THE LINE IS  $y = 0.0868x + 3.0062$ ,  $r = 0.39$

● : NO CORRELATION BETWEEN  $y$  (Na EFFLUX) AND  $x$  (RBC Na)

given by the line  $Y = 0.0868x + 3.0062$ , although the correlation is poor ( $r = 0.39$ ). In hypertensives, there is virtually no correlation between the active sodium efflux and the red cell sodium ( $r = -0.18$ ): The difference in active sodium efflux rate between the hypertensives and normotensives is not statistically significant.

Passive Sodium efflux rate

Passive sodium efflux/rate was not estimated because in the method used, only pump Ia and Ib were blocked. Pump II was not blocked. Hence it was not possible to estimate passive sodium efflux rate by itself. What was obtained was in effect a 'residual' sodium efflux composed of active efflux (Pump II) and passive efflux. Hence only the result of active sodium efflux was presented.

Post Treatment Results (Table XVII, , & )

Sodium content and transport was determined for subjects 1 - 7 of the 47 hypertensive subjects (see appendix), 1 year after the start of treatment. Table XVII A & B summarises the results obtained.

TABLE XVII

1. COMPARISON OF ACTIVE SODIUM EFFLUX IN NORMOTENSIVES  
WITH ACTIVE SODIUM EFFLUX IN HYPERTENSIVES

Subject	Active Sodium Efflux mmol/l/hr.	Difference
Normotensives (16)	5.70 ± 0.53 √2.8 - 10.57	1.47 ± 0.62
Hypertensives (14)	4.23 ± 0.32 √1.90 - 6.07	t = - 2.37 p > 0.01

2. COMPARISON OF EFFLUX RATE CONSTANT IN NORMOTENSIVES  
WITH EFFLUX RATE CONSTANT IN HYPERTENSIVES

Subject	Efflux Rate Constant/hr.	Difference
Normotensives (16)	0.19 ± 0.02	0.08 ± 0.022 t = 3.64
Hypertensives (14)	0.11 ± 0.01	p < 0.001

3. COMPARISON OF POST-STORAGE RBC SODIUM IN  
NORMOTENSIVES WITH THOSE OF HYPERTENSIVES

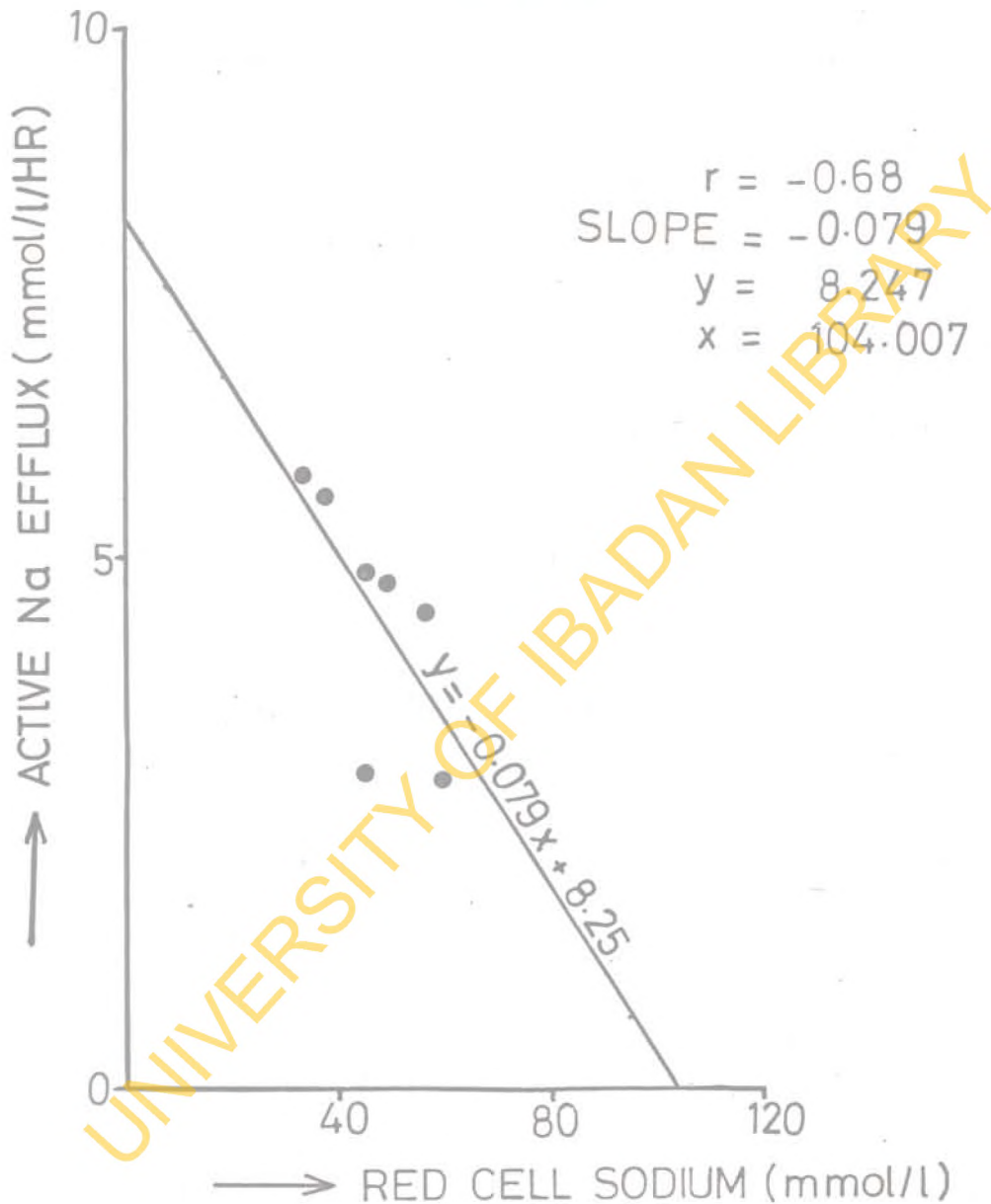
Subject	RBC Na mmol/l	Difference
Normotensives (16)	31.01 ± 2.39	10.59 ± 3.47
Hypertensives (14)	41.6 ± 2.51	t = 3.05 p > 0.01



RBC Na and K Content: In analysing the results, paired t test was used. The pre-treatment value for RBC sodium was  $12.5 \pm 1.88$  mmol per litre, while the post-treatment value was  $12.8 \pm 2.37$  mmol per litre. For RBC potassium, the pre-treatment result was  $83.1 \pm 2.10$  mmol per litre while the post-treatment result was  $83.9 \pm 3.0$  mmol per litre. There was no statistically significant difference between the pre and post treatment values for RBC sodium and potassium.

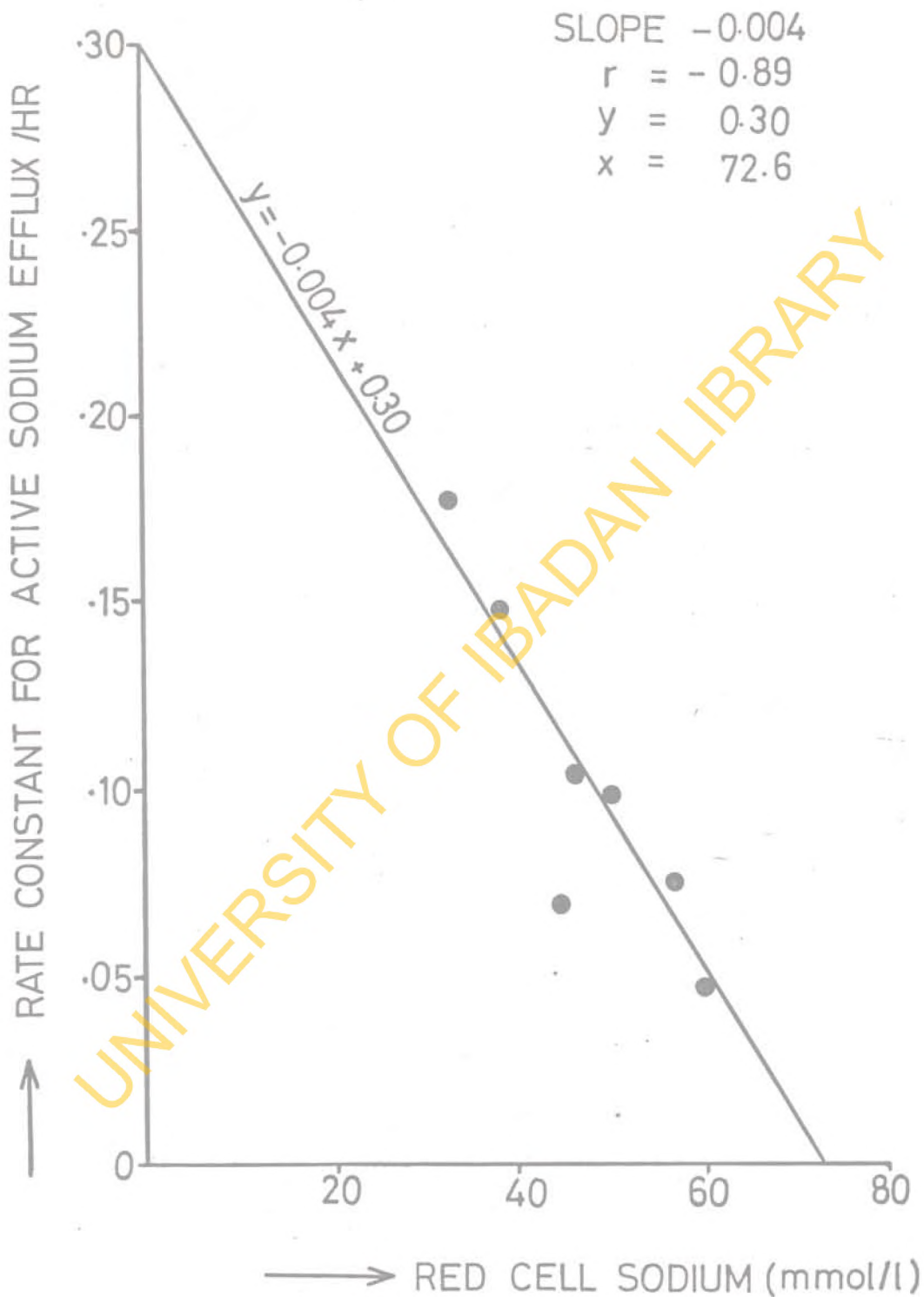
RBC passive Na influx and active Na efflux: The mean post storage sodium content for the 7 hypertensive subjects was  $47 \pm 4.04$  mmol per litre. This was higher than the control post storage sodium of  $31.01 \pm 2.39$  by  $15.99$  mmol/l. This difference is statistically significant. On the other hand, the post storage RBC sodium content 1 year after treatment started did not differ significantly from the pre-treatment value (Tables XVI & XVII). Hence the passive sodium influx into RBC of these 7 hypertensive subjects remained higher than normal in spite of treatment.

FIG. 16



THE RELATIONSHIP BETWEEN THE RED CELL SODIUM AND THE TOTAL AMOUNT OF SODIUM ACTIVELY PUMPED FROM THE CELLS PER HOUR IN HYPERTENSIVES ON TREATMENT FOR 1 YEAR

FIG 17



THE RELATIONSHIP BETWEEN RED CELL SODIUM AND EFFLUX RATE CONSTANT IN 7 HYPERTENSIVE SUBJECTS ON TREATMENT FOR 1 YEAR.

The active sodium efflux rate was  $4.5 \pm 0.47$  mmol/l/hr. This value was again lower than that previously obtained for the 14 control subjects which was  $5.70 \pm 0.53$  mmol/l/hr. The difference was not statistically significant (Table XVIII B). The post-treatment active Na efflux however agrees closely with the pre-treatment value of  $4.23 \pm 0.32$  mmol/l/hr again, there is no statistically significant difference between the 2 (Table XVIII B). The graph of red cell sodium against efflux rate (Fig. 16) shows a linear relationship and a good correlation ( $r = -0.68$ ) between the 2 sets of data in the post-treatment results. In the pre-treatment results, the relationship between the red cell sodium and active sodium efflux was not linear and there was no correlation between the 2 sets of data ( $r = -0.18$ , Fig. 14).

The mean post-treatment efflux rate constant was  $0.10 \pm 0.02$ /hr. while the previously obtained value for controls was  $0.19 \pm 0.02$ /hr. The difference between the 2 was statistically significant. The mean pre-treatment efflux rate constant for these subjects was  $0.11 \pm 0.01$ . There was no



TABLE XVIII

ACTIVE SODIUM TRANSPORT AFTER 1 YEAR OF TREATMENT

(SUBJECTS 1-7 OF TABLE XX, MEAN OF 5 EXPERIMENTS)

A. RBC Na (mmol/l)

	Initial Value	Value 1 Year After Treatment Started.
1.	11.9	11.2
2.	8.9	9.7
3.	12.5	10.6
4.	7.2	10.7
5.	15.1	13.6
6.	24.5	22.4
7.	9.4	9.1
Mean + S.E.M.	12.8 ± 2.37	12.5 ± 1.88

B.

Post Storage Na<sup>+</sup> (mmol/l)

Efflux Rate (mmol/l/hr)

1.	45.0	3.0
2.	60.9	2.9
3.	46.2	4.9
4.	38.2	5.6
5.	57.5	4.5
6.	33.0	5.8
7.	49.7	4.8
Mean + S.E.M.	47.2 ± 4.04	4.5 ± 0.47

C.

Efflux Rate Constant/hr.

1.	0.067
2.	0.048
3.	0.106
4.	0.147
5.	0.078
6.	0.176
7.	0.097
Mean + S.E.M.	0.103 ± 0.018

statistically significant difference between the pre and post-treatment efflux rate constant (Table XVIII). The graph (Fig. 17) of red cell sodium against efflux rate constant 1 year after start of treatment was again linear and the correlation ( $r = -0.89$ ) was better than the pre-treatment value. The difference between the pre-treatment and post-treatment values was not statistically significant, but the difference between the control value and the post-treatment value remained statistically significant.

Problems encountered during the sodium efflux studies

The sodium efflux studies were the most difficult part of the work. The problems encountered centred around the tendency of the stored RBC to lyse during cold storage (at  $4^{\circ}\text{C}$ ) and/or during the 4-hour incubation (at  $37^{\circ}\text{C}$ ).

Briefly stated, if one started with 100 blood samples, 10% lysed during one of the 5 centrifugations associated with changing the cold storage medium. 30% lysed in the course of the 10-day cold storage period. This left 60% to survive till the process of washing of the RBC preparatory

to the sodium efflux studies (Methods, Chap. 2). During the process of washing and centrifugation which followed, another 20% succumbed to lysis leaving only 40% for incubation. At the end of the 4-hour incubation, only 10% (i.e. 1 out of every 4 samples incubated) was useful for estimation of sodium efflux.

I will now break down these problems into the categories enumerated above, and describe what attempts were made to overcome them.

(i) Problems of Cold Storage

In the first 5 days of cold storage, the red cells appeared normal and the cold storage medium remained clear. After 5 days some samples were still haemoglobin-free, but usually a slight reddish tint began to appear, and lysis gradually increased so that at about day 10, 30% were completely lysed. By day 14 up to 80% of the red cells were lysed. Due to this problem of haemolysis, it was decided to cut down the period of cold storage from the 14 days used during preliminary experiments to 10 days. Even after 10 days storage only 60% were suitable for transport studies;

another 10% of the RBC samples having lysed during centrifugation.

(ii) Attempts made to overcome the problem of lysis

The following is an account of attempts made to solve the problem of lysis:

1. Sterile heparinised tubes were used in storing the cells so as to reduce the probability of lysis being due to infection of the red cells. There was no significant differences between the rate of lysis in sterile heparinised tubes and in non-sterile heparinised tubes.

2. Initially, the cold storage medium was prepared in a 1-litre flask, stored in the fridge ( $4^{\circ}\text{C}$ ) and used until the content of the flask was finished. Then it was observed that within a week of storage haemolysis occurred in almost 100% of the stored samples. The pH of the cold storage medium was therefore estimated every 2 days before the medium was added to the red cells. It was observed that the cold storage medium tended to grow more acid with storage.

Subsequently, cold storage medium was prepared in 200 ml flasks and stored frozen ( $-4^{\circ}\text{C}$ ) when not



in use. It was also never stored for more than 72 <sup>at</sup> hours. By so doing the pH was maintained/7.4 ± 0.2. With this precaution, it became possible to reduce lysis to 30% of the stored RBC samples.

3. Each time the cold storage medium was changed, the red cells were removed from the fridge, they were spun, the supernatant was aspirated and fresh storage medium was added to the red cells. This process involved:

- (i) 'thawing' the red cells.
- (ii) heating up the red cells in the centrifuge.
- (iii) subjecting the red cells to the stress of the centrifugal force.
- (iv) cooling the red cells again after the previous thawing and heating.

These 4 processes would not matter much in fresh stored cells, but in cells stored for more than 3 - 4 days, the degree of trauma to the red cells varied directly with period of cold storage. One out of every 10 samples stored for more than 7 days lysed during the process of changing the cold storage medium.

CHAPTER V

THE HYPERTENSIVE PATIENTS -  
CLINICAL AND LABORATORY DATA

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CHAPTER V

THE HYPERTENSIVE PATIENTS - CLINICAL AND  
LABORATORY DATA

1. Mode of referral.
2. Case histories obtained at first attendance.
3. Age distribution.
4. Clinical findings on physical examination.
5. Prevalence of cardiomegaly.
6. Prevalence of retinopathy.
7. Renal status.
8. Prevalence of cardiomegaly and hypertensive retinopathy in patients with high serum creatinine.
9. Comparison of symptoms with severity of hypertension.
10. Follow up.
11. Conclusion.

The series was started with 100 symptomless hypertensive subjects on their first attendance at the medical out-patient clinic. Having established that the patients were physically fit apart from their high blood pressure, blood was obtained from these patients for red cell and plasma electrolytes. The results of these determinations formed the baseline values discussed in chapters III and IV. Relevant data concerning these 100 subjects have also been dealt with at length (Chapter II). So as to ease problems of follow-up, only 47 patients who attended the medical out-patient clinic regularly were followed up, out of the original 100 subjects. These 47 will subsequently be discussed.

#### Mode of Referral.

More than 100 mostly symptomless patients were collected at the rate of 2 - 3 patients per week over a period of 9 months. The question then arose that if these patients were indeed symptomless how did they land themselves in the renal and hypertension clinic? From data obtained during the initial visits of these 47 subjects, it



was established that the following were the sources of referral:

1. The general out-patient department of the UCH, Ibadan, referred 10 subjects who presented with complaints varying from gastro-intestinal disorders (3 subjects) to fainting fit (1 subject).
2. The second, big source of these symptomless hypertensives was the University of Ibadan Jaja Clinic, which referred 10 patients. 2 of these were senior staff being examined for the University's Insurance scheme, the other 8 were members of the junior staff attending the clinic for various purposes.
3. The third big source were the general practitioners who referred 9 subjects. Two of these were diagnosed during insurance medical examinations, and 3 during medical examinations preceding employment, the remaining 4 in the course of routine examinations for other illnesses.

4. The UCH staff clinic supplied 6 subjects, 3 of whom were staff nurses attending the clinic for various complaints, one was a laundry man, another a porter and last a laboratory technician.
5. The surgical out-patient department of the UCH referred 3 subjects, 2 were post-operation follow-up cases, and the third was booked for herniorrhaphy.
6. Three subjects were attending the Eye clinic of the UCH for cataract extraction and 1 subject for poor vision.
7. One patient was referred from the medical out-patient department on account of an old hemiparesis from which he was almost recovered.
8. The remaining 4 subjects were referred with post-partum hypertension from the post-partum clinic of the department of Obstetrics and Gynaecology, UCH.

Case histories obtained at first attendance.

The clinical data on all 47 subjects are summarised in the appendix. Below are outline case histories of 8 of the patients selected to illustrate

different facets of their clinical presentation.

SUBJECT I.

Name: O. S.  
Sex: Male.  
Hospital No: 368328.  
Age: 49 years.  
Occupation: Teaching.  
Weight: 79 kg.  
Height: 5' 9".

Presenting Symptoms: This patient originally presented with symptoms of vertebro-basilar insufficiency, for which he was being investigated, when he was found to have mild hypertension. At the time, he had no symptoms arising from the hypertension per se.

When seen, he was physically fit. He had a cervical collar prescribed for his cervical spondylosis. Apart from this the only other positive finding he had was a supine blood pressure of  $\frac{185}{110}$ . All other findings were normal.

SUBJECT 2.

Name: L. C.  
Sex: Female.  
Hospital No: 85530.  
Age: 49 years.  
Occupation: Staff Nurse.  
Weight: 92 kg.  
Height: 5 ft.

Presenting Symptoms:

This patient was attending the Hospital Staff Clinic because she was depressed, following a bereavement. During the usual physical examination, she was found to have a supine blood pressure of  $\frac{160}{110}$  and was referred to the hypertension Clinic. She had no symptoms referable to her hypertension.

SUBJECT 3.

Name: F. L.  
Sex: Male.  
Hospital No. 249643.  
Age: 47 years  
Occupation: Civil Servant.  
Weight: 80 kg.  
Height: 5 ft. 9 ins.



This patient was a known hypertensive who defaulted for four years, and only turned up this time because he developed a left hemiparesis. He had had no treatment for the four years, and was therefore treated as a new case.

His only complaint was weakness on his left side. On examination, he had a left hemiparesis, and a supine blood pressure of 190/110 was the only other positive physical sign.

SUBJECT 4.

Name:	Y. Y.
Sex:	Male.
Hospital No:	156369.
Age:	50 years.
Occupation:	Labourer.
Weight:	79 kg.
Height:	5' 7"

This patient was discovered to have an elevated blood pressure during consultation for malaria fever at the staff clinic of the University College Hospital, Ibadan. At the time, he had no symptoms referable to his hypertension.

The only positive physical finding was a supine blood pressure of  $\frac{160}{110}$ . As this blood pressure was quite normal for his age, he was not treated initially.

When he was found on X-ray to have a minimally enlarged heart, and his ECG showed left ventricular hypertrophy, it was decided to treat him. On subsequent follow up, he had supine B.Ps. of  $\frac{200}{120}$ ,  $\frac{190}{120}$ .

PATIENT NO. 5.

Name: S. A.  
Hospital No: -  
Occupation: Housewife.  
Sex: Female.  
Weight: 68.2kg  
Height: 5ft. 4".

This 60 year old women presented with joint pains particularly in the knees. In the course of the physical examination, she was found to have mild systolic hypertension and was referred to the clinic. At the time she had no symptoms referable to the hypertension.

On examination, her blood pressure was  $\frac{170}{100}$  which was considered normal for her age. The rest

of the cardio-vascular system was normal on physical examination. In addition, she had polyarthropathy affecting her wrists and the joints of her fingers and her knees.

Investigations showed that she had a positive rheumatoid factor and, in addition she had Osteoarthritic changes in both knees.

PATIENT NO. 6.

Name: Ok. V.  
Hospital No: 379555.  
Age: 20 years.  
Sex: Female.  
Occupation: Housewife.  
Weight: 53 kg.  
Height: 5' 2".

This 20 year old woman presented at the general out-patient department of the University College Hospital 3 months post-partum. She was then complaining of palpitations, loss of energy and tiredness on exertion for 3 months. She was referred to the renal and hypertension clinic.

When seen, she was a small statured but fairly fit-looking woman. She had a severe tachycardia,

pulse 168/min, regular. Her supine blood pressure was  $\frac{160}{100}$ . There were no features of thrototoxicosis. All other physical signs were normal.

PATIENT NO. 7.

Name: G. O.  
Hospital No: 365644.  
Sex: Male.  
Age: 38.  
Occupation: Accountant.  
Weight: 78 kg.  
Height: 5' 7".

This 38 year old patient was found to have hypertension when he presented at the University Health Centre with persistent headache. He was subsequently referred to the hypertension clinic. When seen, he was symptomless. The only positive findings were severe hypertension  $\frac{210}{125}$  supine,  $\frac{220}{140}$  erect, and grade I retinopathy.

PATIENT NO. 8.

Name: U. D.  
Hospital No: 358730  
Sex: Male.



Occupation: University Teacher.  
Age: 35.  
Height: 5' 7"  
Weight: 68.2 kg.

This 35 year old man was seen of the University Staff Clinic where he complained of heaviness in the head. He was then perceived to have a persistently raised blood pressure of  $\frac{180}{120}$  supine. Hence he was referred.

When seen, he was complaining of joint pains and chest pain. He did not then have any symptoms referable to his hypertension. On examination, the only positive findings were a supine blood pressure of  $\frac{180}{130}$  and an apical impulse that was just outside the mid-clavicular line in the 5th intercoastal space.

#### AGE DISTRIBUTION

The ages were volunteered on enquiry by 27 subjects, and were assessed by me in the rest of the 20 subjects. Therefore, at best, the ages are only approximate. The ages varied from 23 to 62 with a mean of  $47.1 \pm 1.4$  years (Table XIX). 70.2% of the subjects studied were in the age group

40 and above, while only 29.8% were in the age group 40 and below. 44.4% of the subjects belonged to the 40 - 50 age group.

TABLE XIX.

Age distribution of 47 hypertensive subjects.

Age (Years)	No. of Subjects	% of Total
10 - 20	-	-
21 - 30	4	8.5
31 - 40	10	21.3
41 - 50	19	40.4
51 - 60	14	29.8

4. Clinical Findings on Physical Examination

Each patient had a thorough physical examination at the first attendance, particular attention being paid to:

1. The cardiovascular system viz: the pulse was checked, any tachycardia being taken into account during treatment. The jugular venous pressure was carefully examined. The blood pressure was recorded both in the supine and erect positions. Cardiac enlargement was also noted as was also the presence of a third heart sound which might indicate impending failure.
2. The fundus was carefully examined for any changes due to hypertension, the fundal appearance being graded from I to IV according to the classification of Keith, Wagener and Barker (1939).
3. The abdomen was carefully palpated for visceral enlargement especially the liver which might indicate right ventricular failure.

5. Incidence of Cardiomegaly

Cardiomegaly assessed by the finding of a clinically displaced cardiac apex, radiological cardiac enlargement or the finding of left ventricular hypertrophy on electrocardiography (ECG) was present in 27 cases. In only 4 of these 27 was the heart clinically enlarged. In all of the 27, the ECG showed left ventricular hypertrophy and in 19 of them, the chest X-ray showed cardiomegaly as well (Table XX).

TABLE XX

Incidence of Cardiomegaly

Type of Investigation	No of subjects with cardiomegaly	% of Total
1. Clinical Examination	4	8.5
2. Chest X-ray	19	40.4
3. E.C.G.	27	57.4

From this it was obvious that the ECG picked up the earliest signs of cardiomegaly much before any radiological signs were apparent. Clinical estimation of cardiomegaly by itself alone would only pick out fairly gross degrees of cardiomegaly.



6. Incidence of hypertensive retinopathy.

Fundal examination was done in 35 cases. The fundi were normal in 12 cases, in 3 cases, the fundi could not be seen on account of cataracts, and in 1 case it could not be seen because the patient was uncooperative. In 19 of the 31 cases whose fundi were visualised, the fundi showed hypertensive retinopathy: 10 grade I, 7 grade II and 2 grade III according to the classification of Keith, Wagener and Barker (1939). These results are shown in Table XXI).

Eight of the patients with retinopathy were male (42.1%) and 11 female (57.9%). This means that retinopathy was more common in the female subjects studied than in the males.

TABLE XXI

Incidence of hypertensive retinopathy

Grade of retinopathy	No. of males	No. of females.
I	3	7
II	3	4
III	2	-
IV	-	-

Of the 19 subjects with retinopathy, 17 (89.5%) had only grade I and II retinopathy. Only 2 subjects (10.5%) had grade III retinopathy, despite the fact that 57% had signs of hypertensive heart disease. This confirms Akinkugbe's observation (1968) that serious degrees of retinopathy are rare even in severe hypertension in Africans.

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7. Renal Status.

All 47 subjects had normal blood urea (Table XXII). All were also initially free of proteinuria. The serum creatinine was normal in all initially, later only in 39 and higher than normal in 8 subjects. The mean values ( $\pm$  S.E.M.) are all shown in Table XXII.

TABLE XXII.

Investigation	N	Mean $\pm$ S.E.M. mg%	Range
Blood Urea	44	26.7 $\pm$ 0.98	19 - 42
Serum Creatinine	41	1.4 $\pm$ 0.07	0.6 - 2.4
Proteinuria	47	Nil.	-

Later, 6 patients developed proteinuria which as determined by albustix (a side-room dip-stick reagent method of testing for albuminuria) varied from trace (1 subject), + (2 subjects); to ++ (3 subjects)

(+ , ++ and +++ represent mild, moderate and severe degrees of proteinuria). These 6 subjects made up 4 of the 8 subjects whose serum creatinine started by being normal and increased to 2mg% and above in the course of their follow up. This change occurred despite adequate control of their blood pressure, (Table XXII).

TABLE XXIII.

Prevalence of cardiomegaly hypertensive retinopathy and proteinuria in patients with high serum creatinine.

Subject No.	Serum	Cardiomegaly	Retinopathy	Proteinuria
(As shown in appendix)	Creatinine			
15 A.V.	2.0	Nil	Nil	Nil
19 O.J.	2.0	Yes	Nil	+
22 O.B.	2.0	Yes	II	Nil
32 E.U.	2.3	Nil	I	+
39 A.G.	2.3	Nil	Nil	Nil
40 G.B.	2.1	Yes	Cataracts	Nil
42 O.A.	2.4	Yes	II	++
47 K.A.	2.0	Yes	I	trace

The 8 subjects (except 1) who had high serum creatinine all fell into the category with moderate to severe hypertension (Chapter II; appendix).

It would seem that there is in these subjects a definite association between the severity of hypertension and the serum creatinine level. Proteinuria also goes hand in hand with a raised serum creatinine



level. For example, of the 47 hypertensive subjects, 7 subjects developed proteinuria. Of these 7, 4 simultaneously or subsequently developed serum creatinine levels of 2 mg% and above. It is not certain whether proteinuria preceded the high serum creatinine or vice-versa; but it would seem that the association of the 2 abnormalities in these 4 subjects is not due to chance.

9. Comparison of symptoms with severity of hypertension.

According to the definitions of mild, moderate and severe hypertensives made in chapter II, 17 of these subjects fell into the mild category, 21 in the moderate and 9 into the severe category, i.e. 36.2%, 44.7% and 19.1% respectively.

Of the mild hypertensives, only 1 patient complained of headache and 1 of palpitations. Of the moderate category, 1 complained of headache, 1 of dizziness and 1 of loss of consciousness. Of the severe hypertensives, 1 complained of poor vision and 1 of dyspnoea. Thus it was observed that only 11.8%, 14% and 22.2% respectively of the mild, moderate and severe hypertensives had any symptoms

at all. This was in spite of the fact that at the time of diagnosis, 57.4% of these patients had signs of hypertensive heart disease; and 17% later showed signs of renal involvement.

More than half the patients (57%) when first seen had cardiomegaly (Table XX) as evidenced by clinical, radiological and electrocardiographic examination. This feature was also observed by Oviasu (1974) who found that this was the major cause of death in hypertension in a similar group of Nigerian subjects.

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### Result of Follow-up

At first attendance as mentioned earlier, 27 (i.e. 55%) of the hypertensive subjects already possessed evidence of hypertensive heart disease.

During the period of follow-up, 4 of these patients (subject 10, 22, 27 and 29) went into left ventricular failure and have been digitalised for periods varying from 6 months to 2 years.

Subjects 15, 19, 22, 32, 39, 40, 42 and 47 developed albuminuria and their plasma creatinine have risen to 2mg% and above.

Two subjects (subjects 12 and 24) suffered cerebro-vascular accidents. Subject 12 suffered a major cerebro-vascular accident and died, while subject 24 suffered a minor one and survived with a left hemiparesis.

The 33 patients not yet mentioned are alive and well. They have not suffered any further deterioration in their cardio-vascular status, since treatment started.

### Conclusion

The 47 hypertensive subjects discussed in this chapter were followed up for periods varying from 1 to 4 years. All except subjects 42 and 47 responded with adequate lowering of their blood pressures both systolic and diastolic. Subjects 42 and 47 were more difficult to control as their diastolic pressures remained persistently raised (120 - 140 mm Hg). Nevertheless, 4 patients went on to develop renal decompensation and 2 had cerebrovascular accident. Before treatment even started, 27 subjects already had evidence of hypertensive heart disease, so it is expected that more subjects will probably succumb to left ventricular failure.

These results are in agreement with those of Oviasu (1974) who found in another group of Nigerian hypertensives that 50% have complicated disease which terminate in heart failure, cerebrovascular accident or renal failure.

All the same, it is encouraging that 33 of the 47 subjects are alive and well. One may therefore venture to say like Oviasu (1974) that in uncomplicated disease, the prognosis for hypertensives in



Nigerians is good provided that meticulous care is taken with the drug regimen. Nigerians still have the advantage of a relative absence of ischaemic heart disease.

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CHAPTER VI

DISCUSSION

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### Methods of Study

Measurements of RBC cation content have, in the past, used 2 main approaches:

1. The indirect method.
2. The direct method.

#### The indirect Method - (Czackes et al., 1963):

Here the water and electrolyte content of whole blood and of plasma are first determined. The haematocrit is then determined. RBC sodium content (Nac) and potassium content (Kc), are then calculated from the results obtained by deducting the results for plasma from that of whole blood.

This method suffers from two inaccuracies:

- (1) The Nac is higher than results obtained for washed cells, because trapped plasma in the packed cell column contains Na.
- (2) The buffy coat on the top of the red cell column, also accounts for some inaccuracy in the Kc determination.

#### The Direct Method of Measuring RBC Na and K

This method is divisible into 2 :-

- (1) One method utilizes packed unwashed cells (Beilin, et al., 1966; Valberg et al., 1965).
- (2) The other utilizes packed and thoroughly washed cells (Smith and Samuel, 1970; Kurantsin-Mills,

Kudo and Addae, 1974).

(i) Here the given sample of blood is packed, and the haematocrit is determined. Plasma and the buffy coat are removed. The sample for Na and K determination is then taken from the bottom of the packed cell column, and lysed in deionised water for flame photometry. RBC Na and K are then calculated with the aid of the haematocrit.

(ii) In the second direct method, the blood is again packed. Serum and the topmost layer of cells are removed. The cells are then washed in an appropriate buffer medium and re-packed. The washing is done 3 times after which the packed cells are re-suspended in the buffer medium. The haematocrit (PCV) of the re-suspended red cells is determined, and a sample of the re-suspended cells is lysed for flame photometry. RBC Na and K are calculated with the aid of the PCV.

The big advantage of washed cells over unwashed cells in the direct method lies in the fact that with washed cells, contamination of the red cells with plasma Na is reduced to a minimum. Hence, the RBC Na results obtained are much more reliable than when unwashed cells are used. Washed cells also



have their own disadvantages (Valberg, Holt, Paulson and Szivek, 1965), which will be discussed later.

A difficulty common to both methods, of course, is that even after prolonged centrifugation at high speed, some plasma or wash solution always remains trapped between the RBCs. This gives rise to false values, especially for Na which is in a much higher concentration in plasma than in RBCs (Vasquez, Newerly, Yalow and Berson, 1952). This error would appear to be greater in the case of unwashed cells than in washed cells.

The evaluation of trapped plasma as this plasma error is commonly called, has been the subject of numerous studies for several years. The magnitude of the plasma error has been determined with substances which do not enter the RBC, such as Evan's blue (Chaplin & Mollison, 1952; Kurantsin-Mills, Kudo and Addae, 1974), radio-iodinated human serum albumin ( $^{131}\text{I}$  HSA) and radio-sodium ( $^{24}\text{Na}$ ) (Vasquez, Newerly, Yalow and Berson, 1952).  $^{14}\text{C}$  has also been used as marker in estimating plasma Na trapping (Beilin, Knight, Munro-Faure and Anderson, 1966). This method was developed when it was found that iodinated human

serum albumin ( $^{131}\text{I}$ .HSA) under-estimates the amount of trapped extra-cellular Na in the packed cell layer.

Valberg et al., (1965) did spectrochemical analysis of sodium, potassium, calcium, magnesium, copper and zinc on red cells of fifty normal human subjects.

They found that:

1. Determination of erythrocyte sodium from whole blood preparations is inaccurate and normal values reported with this method are unusually high.

2. The effect of washing erythrocytes with magnesium chloride or tris-choline buffer leads to elusion of some of the cations from the red cells particularly sodium and calcium. Hence, lower values than normal are obtained for these two ions from washed cells.

3. When trapped plasma corrections are applied, the correction should be determined for each individual sample. This gives a closer range of results and consequently a lower standard deviation. Where trapped plasma corrections are made from a composite calibration curve, the standard deviations and range

of results are usually much higher than in those experiments in which trapped plasma is calculated from individual calibration curves.

In this study, the method used to determine red blood cell sodium and potassium was the direct method, the cells being washed and correction made for trapped plasma. In making correction of trapped plasma, the Evan's blue method was used. A similar method for correcting for trapped plasma was used by Chaplin & Mollison (1952) and by Kurantsin-Mills et al. (1974). The mean value for trapped plasma obtained from 7 normal subjects in this study was 3.2%, a value very similar to that obtained in normal Ghanaians by Kurantsin-Mills et al. (1974). It is also not very dissimilar from the value of 2.6% obtained by Chaplin & Mollison (1952) in their normal white subjects. The mean value obtained from these seven subjects was used to correct for trapped plasma in all the 908 normal subjects whose erythrocyte sodium was determined. According to Valberg et al. (1965), this could lead to wide inter-individual variations.



in the values of the erythrocyte sodium. Nevertheless it was not considered practicable for technical reasons to determine the trapped plasma for individual samples. However, in view of the large numbers involved and also in view of the close similarity between the mean values for trapped plasma in this study and in early studies, it is not considered likely that the error that might be introduced by the use of a mean value to correct for trapped plasma will be sufficiently large to affect conclusions drawn from the results obtained.

Comparison of result obtained for caucasians  
In Nigeria with results for caucasians elsewhere.

Erythrocyte sodium and potassium content and transport have been more extensively studied in caucasians than in negroes. Results obtained in my hands on Nigerians may differ from results obtained in caucasians from studies in other laboratories not necessarily because of inter-racial differences, but because of the well-known changes that may arise as a result of subtle differences in techniques in different laboratories. In order to evaluate the



TABLE XXIV

COMPARISON OF RBC SODIUM, POTASSIUM AND WATER CONTENT  
OF CAUCASIAN CONTROLS IN NIGERIA WITH THOSE OF  
CAUCASIAN CONTROLS OF OTHER WORKERS

A.	Subject	RBC Na <sup>+</sup> mmol/l	RBC K <sup>+</sup> mmol/l	RBC Water %
	1. Caucasians in Nigeria (7)	6.7 ± 0.83	86.7 ± 3.4	64.0 ± 0.98
	2. Czackes et al., (1963) (10)	7.8 ± 0.16	93.0 ± 1.10	54.8 ± 0.13
	3. Beilin et al., (1966) (10)	6.8 ± 0.55	91.73 ± 1.90	54.12 ± 2.15
	4. D'Amico (1958) (25)	18.37 ± 6.49	95.96 ± 3.99	-----
	5. Smith & Samuel, (1970) (60)	7.04 ± 0.18	102.4 ± 0.74	62.0 ± 0.36
	6. Valberg et al., (1965) (50)	12.91 ± 1.91	136.08 ± 23.0	67.9 ± 2.2
B.	Difference			
	1 vs 2	1.1 ± 0.85	6.3 ± 3.57	0.8 ± 0.99
	t	1.29; > 0.1	1.76; > 0.05	0.80; > 0.1
	1 vs 3	0.1 ± 0.99	5.03 ± 3.89	0.12 ± 2.36
	t	0.10; > 0.5	1.29; > 0.1	0.05; > 0.5
	1 vs 4	11.67 ± 6.54	9.26 ± 5.24	-----
	t	1.78; > 0.05	1.77; > 0.05	-----
	1 vs 5	0.34 ± 0.85	15.7 ± 3.48	2.0 ± 1.04
	t	0.4; > 0.5	<u>4.5; &lt; 0.001</u>	1.92; > 0.05
	1 vs 6	6.21 ± 2.08	49.38 ± 23.25	3.9 ± 2.41
	t	<u>2.99; &lt; 0.01</u>	2.12 > 0.01	1.62; > 0.1

Underlined: Statistically significant differences.

magnitude of this difference attributable to difference in the laboratories of investigation, seven caucasians working in the same area of Nigeria from which the local population was drawn were investigated with a view to comparing their results with those of other caucasians (Table XXI).

The mean erythrocyte sodium, potassium and water obtained for the seven caucasian controls in this work were 6.7 mmol/l, 86.7 mmol/l and 64.0% respectively (Table XXI).

Czackes et al. (1963) used unwashed red cells and found in 20 caucasian subjects (Hebrews) a mean RBC sodium of 7.8 mmol/l, potassium of 93 mmol/l and water of 64.8%. Beilin et al. (1966), using a different technique from the one used here, and determining individual trapped plasma which they then used to correct for RBC sodium in each case, found in 10 subjects the following results:- RBC sodium of 6.8 mmol/l potassium of 91.73 mmol/l and water of 64.12%.

D'Amico (1958) determined RBC sodium and potassium in 25 control subjects from whole blood preparations. He had sodium and potassium values of 18.37 and

95.96 mmol/l respectively. Czackes, Aviram, Keynan and Ullman (1967) found in 32 control subjects, RBC sodium, potassium and water of 7.8 mmol/l, 93.0 mmol/l and 64.8% respectively. These workers also used unwashed cells. This result was identical to a previous one by Czackes et al. (1963). Smith and Samuel (1970) using washed red blood cells found in 60 normal control subjects a mean RBC sodium of 7.04 mmol/l, potassium of 102.4 mmol/l and water of 62%. Valberg et al. (1965), using unwashed red blood cells in a study involving 50 normal subjects, found mean values of 8.79 mmol/l, 92.67 mmol/l and 68.13% respectively for RBC sodium, potassium and water.

From the above results (summarised in Table XXIV) it is observed that the values for RBC sodium obtained in this work agree very closely with those of Czackes et al. (1963), Czackes et al. (1967), Beilin et al. (1966) and Smith and Samuel (1970). It is very much lower than that of D'Amico (1958) but the difference is not statistically significant since the standard error D'Amico obtained was very large. It is significantly lower ( $P < 0.01$ ) than that of Valberg, et al. (1965).



The big differences between this result and that of D'Amico could be attributed to D'Amico's use of a whole blood preparation since it has been established that the determination of erythrocyte sodium from the difference between plasma and whole blood sodium is inaccurate and normal values reported with this method are unusually high (Valberg et al., 1965).

The caucasian RBC sodium is also significantly lower than that of Valberg et al. (1965) because washed cells were used in this work whereas Valberg et al. used unwashed red blood cells. As pointed out by Valberg et al. (1965), washing of red cells leads to elution of the intracellular ions especially sodium and calcium so that very low values are obtained for these ions.

The value obtained for the caucasian RBC potassium showed no significant difference from those of Czackes et al. (1963), Czackes et al. (1966), Beilin et al. (1966) and D'Amico (1958). Though the caucasian RBC potassium value is very much lower than that for Valberg et al. (1965), the difference is not statistically significant. It is also significantly



lower than that of Smith and Samuel; (1970).

There is no statistically significant difference between the red cell water of the caucasians in this study and those of the above five groups of workers as shown in Table XXIV.

From the foregoing, it is clear that the values obtained for red blood cell sodium, potassium and water in caucasians living in Nigeria do not differ significantly from values obtained for caucasians from studies in other parts of the world. Where differences have been found, such differences could be easily explained from differences in the methods used to determine the various parameters. On the basis of these results, it can be concluded that no significant differences are to be expected solely on account of different laboratory locations when comparing my results with those of other workers.

Comparison of results for 908 Nigerian controls

with those of Caucasians in Nigeria.

[Table XXV]

RBC sodium, potassium and water obtained for the 908 controls who were Nigerians were 9.7 mmol/l, 88.0 mmol/l and 65.2% respectively. The seven caucasians included in the series had 6.7 mmol/l, 86.7 mmol/l and 64% respectively of RBC Na, K and red cell water. Of these values only the RBC sodium differed significantly ( $P < 0.01$ ) in the two groups of subjects. There is no significant difference in the red cell potassium nor in the red cell water.

∴ Differences in the red cell sodium of negroes and caucasians have also been recorded by other workers. For example, Kurantsin-Mills, Kudo and Addae (1974) recorded mean values of 13.96 mmol/l and 99.07 mmol/l for sodium and potassium respectively in 47 control Ghanaians and 9.00 mmol/l and 95.34 mmol/l for sodium and potassium respectively in 8 caucasians. The red cell water for Ghanaian subjects was 66.85%, they did not determine red cell water for the caucasians.

TABLE XXV

COMPARISON OF RBC SODIUM, POTASSIUM AND WATER CONTENT  
OF NIGERIAN CONTROLS WITH THOSE OF THE  
7 CAUCASIAN SUBJECTS IN THIS STUDY

Subject	RBC Na <sup>+</sup> mmol/l	RBC K mmol/l	RBC Water
1. Nigerians (908)	9.7 ± 0.13 [2.0 - 28.2]	88.0 ± 0.30 [68.1 - 118.7]	65.2 ± 0.21 [50-71.9; 71]
2. Caucasians 7 subjects	6.70 ± 0.83 [2.4 - 8.6]	86.7 ± 3.4 [75.3 - 100.4]	64.0 ± 0.98 [62.1-65.4; 3]
Difference	3.0 ± 0.84	1.3 ± 3.41	1.2 ± 1.00
t	3.57	0.38	1.2
p	0.001	> 0.5	> 0.1

Comparison of the RBC sodium, potassium and water of the Nigerians with those of other blacks. (Table XXVI, XXVII)

The mean red cell sodium, potassium and water of 9.7 mmol/l, 88.0 mmol/l and 65.2% respectively obtained in Nigerians were all lower than those of Kurantsin-Mills et al. (1974) who had 13.96 mmol/l, 99.07 mmol/l and 66.85% respectively (Table XXVI). The difference between the red cell sodium is statistically significant ( $P < 0.01$ ). The difference between the red cell Potassium is also statistically significant. The difference between the cell water is not statistically significant.

Ezeilo (1972) obtained RBC sodium of 20.1 mmol/l, potassium 149.0 mmol/l and red cell water of 66.9%. The results for his sodium and potassium were significantly different ( $P < 0.01$ ) from the ones reported here. There was no significant difference between his RBC water and that obtained here (Table XXVII).

Balfe et al. (1968) also reported an undefined "hereditary sodium transport defect" in a black American family of 32 in which the mean RBC sodium was 14.4 mmol/l. Their normal control value in this work



TABLE XXVI

COMPARISON OF RBC SODIUM POTASSIUM AND WATER CONTENT OF  
 NIGERIAN CONTROL SUBJECTS WITH THOSE OF CALCASIAN  
 IN NIGERIA AND GHANAIS OF KURANTSIN-MILLS ET AL., (1974)

Subjects	RBC Na	RBC K	RBC Water
1. 908 Nigerians	9.7 $\pm$ 0.13 [2.0 - 28.2]	88.0 $\pm$ 0.30 [68.1-118.7]	65.2 $\pm$ 0.21 [50-71, 71]
2. 47 Ghanaians	13.96 $\pm$ 0.76 [9.40-27.21]	99.07 $\pm$ 1.19 [74.10-124.10]	66.85 $\pm$ 0.46 [64.63-69.92]
3. 7 Caucasians	6.70 $\pm$ 0.83 [2.4 - 9.6]	86.7 $\pm$ 3.4 [75.3 - 100.4]	64.0 $\pm$ 0.98 [62.1-65.4; 3]
Difference 1 vs 2	4.25 $\pm$ 0.77	11.07 $\pm$ 1.23	1.65 $\pm$ 0.51
t =	5.5 < 0.001	9.0 < 0.001	3.24 < 0.001
1 vs 3	3.0 $\pm$ 0.84	1.3 $\pm$ 3.4	1.2 $\pm$ 1.00
t =	3.57 < 0.001	0.38 > 0.5	1.2 > 0.1

In parentheses: range, number of subjects.

TABLE XXVII

COMPARISON OF RBC SODIUM POTASSIUM AND WATER CONTENT  
OF NIGERIAN CONTROLS WITH THOSE OF OTHER BLACKS

Subject	RBC Na <sup>+</sup> mmol/l	RBC K <sup>+</sup> mmol/l	RBC Water
1. Nigerians (908)	9.7 ± 0.13 (2.0 - 28.27)	88.0 ± 0.30 (58.1-118.77)	65.2 ± 0.21 (50.0-71.9; 71.7)
2. Ghanaians (Kurantsin-Mills et al., 1974; (47)	13.96 ± 0.76 (9.40 - 27.217)	99.07 ± 1.19 (74.10-124.107)	66.85 ± 0.46 (54.63-69.927)
3. Zambians (Ezeilo, 1971) (133)	13.47 ± 6.2 ---	99.8 ± 6.2 ---	66.9 ± 3.8 ---
4. Tosteson (1955)	10.8	91.4	---

was 7.6 mmol/l, but it is not certain whether the normal controls were drawn from black or white populations.

The differences between the results for RBC sodium and potassium obtained in this work and those of Ezeilo (1972) were attributable largely to differences in methodology. Ezeilo did his estimations on whole blood preparations. This method has a wide margin of error (Valberg et al., 1965) both for the RBC sodium and potassium. The sodium error is derived from the trapped plasma while the potassium error is derived from the buffy coat.

The difference between these results and those of Kurantsin-Mills et al. (1974) is easily explained. It cannot be due to the method of preparation of the RBC since washed cells were used in both studies. The details of the techniques used in both were also similar. The percentage trapped plasma for the Ghanaian series was 2.1 while mine was 3.2%, a difference of 1.1%. This might account in part for my lower red cell sodium, but is too small a difference to account for the whole of the big difference in RBC sodium. (Table XXVI).

TABLE XXVIII

COMPARISON OF PBC SODIUM CONTENT IN NORMOTENSIVES  
WITH HB. GENOTYPES AA, AS AND AC

Subjects	Na mmol/l	Difference	p Value
1. AA	9.6 ± 0.15 (2.4-24.7; 633/	1 vs 2 0.1 ± 0.308	0.32 p > 0.50
2. AS	9.7 ± 0.27 (3.1-23.3; 211/	2 vs 3 1.1 ± 0.81	1.35 p > 0.1
3. AC	10.8 ± 0.75 (3.4-28.2; 50/	1 vs 3 1.2 ± 0.77	1.56 p > 0.1

In parentheses: range, number of subjects.



TABLE XXIX

## COMPARISON OF RBC POTASSIUM CONTENT IN NORMOTENSIVES

WITH HB. GENOTYPES AA, AS AND AC

Subjects	RBC K mmol/l	Difference	t
1. AA	87.9 $\pm$ 0.32 /69.1-118.7; 633/	1 vs 2 = 0.8 $\pm$ .75	1.07 p > 0.1
2. AS	88.7 $\pm$ 0.69 /70.0-113.0; 211/	2 vs 3 = 2.1 $\pm$ 1.45	1.45 p > 0.1
3. AC	86.5 $\pm$ 1.29 /73.2-117.2; 50/	1 vs 3 = 1.3 $\pm$ 1.32	0.98 p > 0.1

Environmental factors cannot account for differences in the results since both sets of subjects belong to the same kind of hot, humid, environments. The reasons would probably lie in the fact that the RBC sodium and potassium results for Kurantsin Mills et al. were expressed per litre of red cell water, whereas my results were expressed per litre of erythrocytes. Since red cell water averaged 66% in both studies, results for Kurantsin Mills et. al. would be 34% higher than mine. Had these workers expressed their results per litre of erythrocytes, there would be no statistically significant difference between their results and mine.

Within group Comparisons. (Tables XXVIII & XXIX)

The results obtained for RBC sodium, potassium and water for the normotensive group were further analysed according to the haemoglobin genotypes (Tables XXVIII & XXIX). It was shown that there was not statistically significant difference in the red cell sodium, potassium and water content of subjects with haemoglobin genotypes AA, AS and AC. Subjects with haemoglobin genotypes SS or SC were not included in this study.

From these results it would seem that for purposes of RBC sodium and potassium content, cells with haemoglobins AS and AC behave like those with haemoglobin AA. Even RBC with haemoglobin SS have normal sodium and potassium content and transport when maintained in the disc form by incubation in oxygen (Tosteson et al., 1955). It is only when they are

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sickled as in conditions of reduced oxygen tension that they have RBC sodium and potassium content and transport above normal values (Tosteson et al., 1955). Kurantsin-Mills et al. (1974) also reported that in sickle-cell haemoglobin SS disease, the RBC sodium is 40% higher and potassium 10% lower than in Hb. AA cells. They further said that under the experimental conditions employed, not less than 85% of their red cells appeared biconcave. This seemed to contradict the findings of Tosteson et al. (1955), which claimed that when the cells were biconcave, the sodium and potassium content and transport were normal. The two findings can probably be reconciled if one might make the observation that the RBC of Kurantsin-Mills et al., though initially biconcave discs assumed the sickled forms during the experimental procedure.

According to Tosteson et al. (1955), two properties of red cells of patients with SS and SC disease are known to depend on oxygen tension of the suspending medium:

1. the shape.
2. the sodium and potassium content and transport.

Tosteson et al. (1955) have also shown that these two properties are independent of oxygen tension in the red cells of subjects with AA and AS genotypes.



From the results for sodium and potassium content obtained for haemoglobin AA, AS and AC in this work it was concluded that sodium and potassium contents are identical in the 3 genotypes. Hence at least for purposes of RBC sodium and potassium content, cells with AS and AC haemoglobins behave like those with normal haemoglobins.

Normotensives compared with hypertensives (Table IV).

The mean erythrocyte sodium, potassium and water obtained for the 100 hypertensive subjects in this study were 13.5 mmol/l, 90.9 mmol/l and 69.4% respectively. These are all significantly higher than those of the normotensive group which were 9.7 mmol/l, 88.0 mmol/l and 65.2% respectively. The difference is greatest in the case of the RBC sodium which is 39.2% higher than the normotensive result, while the potassium and RBC water are higher by 3.3% and 6.4% respectively.

Other workers have also found an increase particularly in intracellular sodium content in hypertension. Edmondson et al. (1975) found increases in sodium and water content of the white blood cells in essential hypertension. D'Amico (1958) observed

increased red cell sodium in essential hypertensives who were not in failure. From those in failure, he observed much higher increases in sodium and in the most severe cases, he also observed that there were increases in RBC potassium. He did not distinguish right from left-sided failure since he obtained the same result from both.

Many attempts have been made in the past to demonstrate abnormal sodium and potassium metabolism in hypertension, and to find a cause and effect relationship between the two (Tobian and Binion, 1952; Tobian and Binion, 1954; Tobian and Redleaf, 1958; Grollman, 1954).

Tobian and Binion (1952) found an increased sodium and water content in the renal arteries of hypertensive subjects. There have also been reports of increased sodium and potassium in the blood vessels of animals with experimental hypertension, (Tobian and Redleaf, 1958; Tobian and Binion, 1954; Grollman, 1954).

The consistently higher values for these two cations in hypertension, no matter how induced, led Tobian and Redleaf (1958) to presume "a chemical

lesion in the arterial wall, which may be important in the pathogenesis of the increased peripheral resistance characteristic of hypertension." This assumption remains to be proved. Hence, the mechanism by which changes in ionic composition of the aorta, or the circulating red cells might modify sympathetic tone in the blood vessels, remains speculative.

Conceivably, such changes might alter the tone of arterial smooth muscle, either through an effect on membrane potential or through a direct effect on actomyosin. An increase in the intracellular concentration of potassium would tend to increase membrane polarization.

On the other hand, ionic composition influences the contractile properties of actomyosin in glycerol-extracted preparations of skeletal muscle in which polarization of the membrane no longer exists (Weber and Portzehl, 1954).

Hadju (1953) had noted that the total amount of potassium per unit of muscle mass in the frog heart is related to the strength of contraction. Also, the use of a perfusing medium low in potassium alters the contractile response of arterial strips (Bohr, Brodie and Cheu, 1957, Leonard, 1957).



Hence from the above evidence, we can only speculate on the importance of the ionic changes in the red cells and large blood vessels of the hypertensive subject. It remains to be demonstrated whether such changes occur in the arterioles, and if they do, whether or not they would enhance contractility requires further clarification.

Influence of age and sex on RBC sodium and potassium in normotensives and hypertensives

(Figure 5, Table V, VII(i & ii)).

Red Cell Sodium

In both sexes red cell sodium in the controls varied very little with age until age 50 when the level fell steadily to reach a minimum level of 6 - 7 mmol/l at age 60. There was no significant sex difference in red cell sodium.

In the hypertensives, there was no significant sex difference in the red cell sodium. The highest concentrations of red cell sodium were found at ages 30 and 65 in hypertensives, and the lowest at age 50.



Beilin et al. (1966), found that RBC Na was not affected by age in men, but in women concentrations in middle age were higher than in youth. There are no such comparisons in hypertensives in the literature. Red Cell Potassium (Fig. 6(1 & 2)).

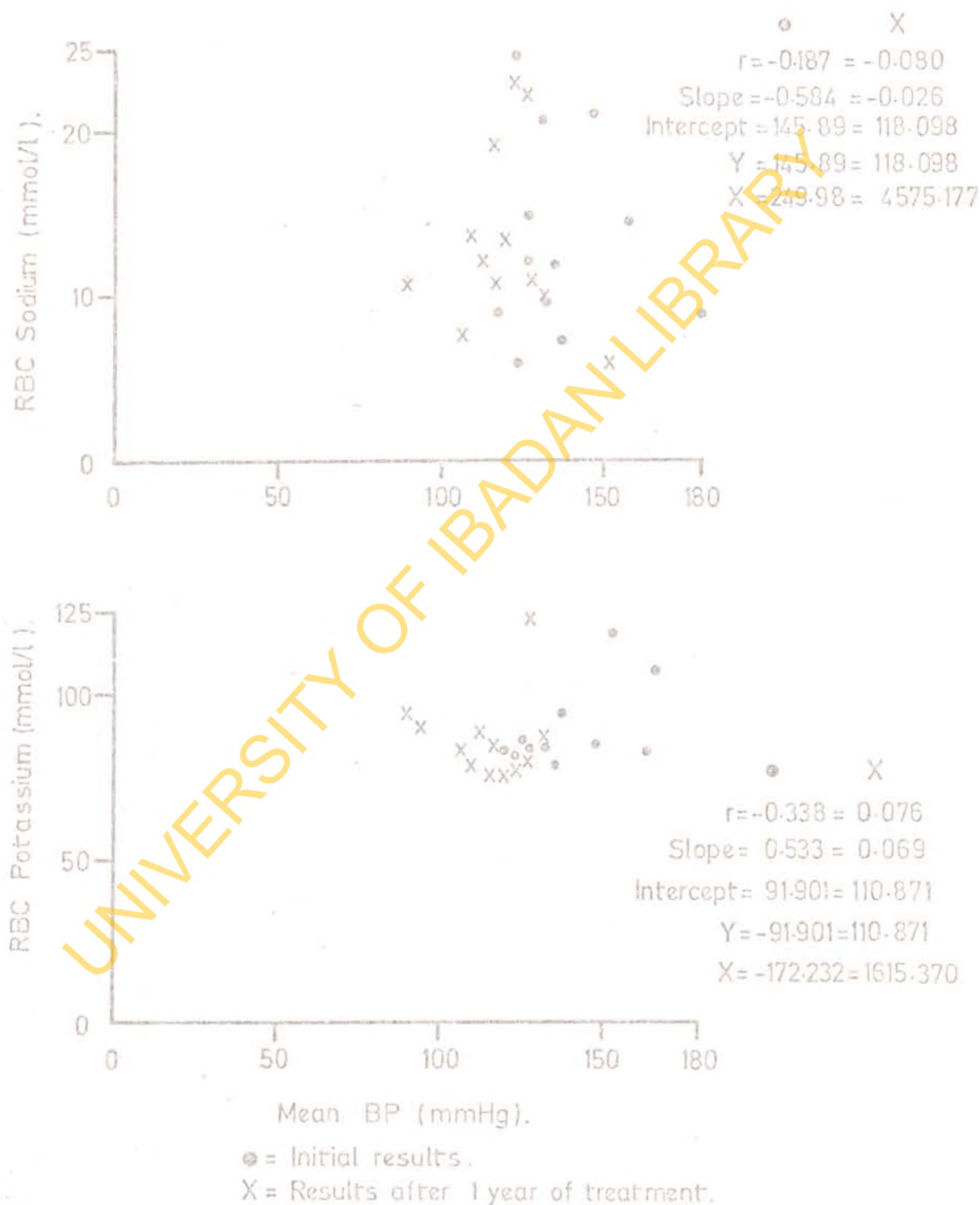
In the controls, the red cell potassium for males was identical with that for females. Apart from a spike which occurs between ages 19 - 24, RBC potassium appears to be constant and to vary very little with age until age 40 when a progressive fall with age sets in reaching a minimum of 78 mmol/l at age 60 years. The reason for this is not clear.

In hypertensives, there is an initial rise in RBC potassium leading to a peak of 94 mmol/l at age 25 years and falling to a minimum value of 84 mmol/l at about age 35 years. A second rise occurs with a peak at age 55 before a final falling off.

The two peaks for RBC sodium and potassium in the hypertensive subjects are very close, those for sodium occurring at ages 30 and 65 and those for potassium at ages 35 and 55 respectively. More than half the hypertensive patients seen in the course of this study have ages ranging from 30 - 55 years. Those with the highest levels of blood pressure recordings also lie

FIG. 15

Relationship between mean blood pressure and red cell sodium and potassium.



in this age group. One cannot but wonder whether the degree of abnormality in the RBC sodium and potassium might not be related to the level of blood pressure reading. It was in order to test this possibility that mean blood pressure was plotted against red cell sodium and potassium (Figure 15). These plots did not show any good correlation between the height of blood pressure and intracellular sodium or potassium.

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Plasma Sodium and Potassium Content (Table XXX).

1. Caucasian Results in this work compared with Caucasian Results elsewhere.

The mean plasma sodium for the 7 Caucasians in this work was 129 mmol/l, the mean plasma potassium was 4.5 mmol/l. Roberts (1967) carried out a survey on 400 blood donors in Birmingham in whom he analysed 17 blood constituents each, using an autoanalyser. His values for plasma sodium and potassium were  $130 \pm 2.5$  (134 - 143) mmol/l and  $4.0 \pm 0.15$  (3.6 - 4.7) mmol/l respectively. The determination was done on 246 and 233 subjects for sodium and potassium respectively.

The figures obtained for caucasians in this study using a manual procedure with an Eel flame photometer were similar to those of Roberts (1967).

2. Caucasian results in this work compared with Nigerian results in other studies.

The mean plasma sodium and potassium of 129 mmol/l and 4.5 mmol/l obtained for the 7 caucasians compare very favourably with those for the normal subjects who had 128 mmol/l and 4.6 mmol/l respectively.

Edozien, (1958) reported values of 136 - 150 mmol/l and 4.5 - 5.5 mmol/l for serum sodium and potassium



TABLE XXX

COMPARISON OF PLASMA SODIUM AND POTASSIUM OF  
NIGERIANS WITH THOSE OF CAUCASIANS

Subject	Plasma Na	Plasma K
1. Caucasians in this work	129 + 1.70 [122-135; 7]	4.8 + 0.12 [4.2 - 5.1; 7]
2. Caucasians of Roberts (1966)	130 + 2.5 [134 - 143; 233]	4.0 + 0.15 [3.6 - 4.7]
3. Nigerians in this work	128 + 0.50 [95 - 155; 209]	4.6 + 0.08 [2.5 - 5.5; 200]
4. Nigerian Hospital population (McFarlane et al. 1970)	130 + 5.2 [119 - 140; 948]	3.5 + 0.8 [1.9 - 5.1; 963]
5. Nigerians (Edozien, 1958)	mean unknown [136 - 150]	mean unknown [4.5 - 5.5]
6. Nigerian Medical Students (Salako, 1971)	138 + 3.1 [range unknown; 24]	4.7 + 0.48 [range unknown; 24]
B. 1 vs 2	1 + 3.02 t = 0.33 p > 0.5	0.8 + 0.19 t = 4.2 p < 0.001
1 vs 3	1 + 1.77 t = 0.56 p > 0.5	0.2 + 0.14 t = 1.43 p > 0.1
3 vs 4	2 + 5.22 t = 0.383 p > 0.5	1.1 + 0.80 t = 1.38 p > 0.1
3 vs 6	10 + 3.14 t = 3.18 p < 0.01	0.1 + 0.49 t = 0.20 p > 0.5

In parentheses: range, number of subjects.

respectively. His methodology was similar, and his controls were drawn from the same population as mine.

MacFarlane et al. (1970) also worked on a similar hospital population of subjects at University College Hospital, Ibadan. Their values of 130 mmol/l (n = 948) and 3.52 (n = 963) mmol/l respectively for plasma sodium and potassium were closely comparable to the results obtained here. Their Na value was 2 mmol/l higher than mine, and their potassium was 1.08 mmol/l lower. The differences in both cases were not statistically significant (Table XX).

Salako (1971) also obtained mean values of  $138 \pm 3.1$  and  $4.7 \pm 0.48$  in 24 volunteer medical students. His sodium values were again close to those of Edozien but higher than mine and those of McFarlane et al. (1970); the difference of 10 mmol/l is statistically significant. The values he obtained for the plasma potassium were not significantly different.

From the foregoing, it is obvious that even within the University College Hospital, Ibadan, there is as yet, no general agreement as to what constitutes normal values in these plasma electrolytes. Edozien

(1958) and McFarlane et al. (1970), worked in the same laboratory and obtained different results for their plasma sodium and potassium.

There was no significant difference between the results of McFarlane et al. (1970) and my results. The results of Salako (1971) differed significantly from mine only in the plasma sodium for his normal group of subjects. His normal group, I think, was biased because he used medical students. Medical students cannot be regarded as being representative of the community at large, because Edozien (1958) has shown that their biochemical data tend to be different from those of the general population.

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Plasma sodium and potassium contents:  
normotensives compared with hypertensives

(Table XXXI)

The mean plasma sodium and potassium obtained for the normotensive group were 128 mmol/l and 4.6 mmol/l while the values for the hypertensives were 138 mmol/l and 3.7 mmol/l respectively. Thus the sodium value for the hypertensive subjects is 10 mmol/l higher than that for the normotensive. This difference is statistically significant and at variance with the observation of Salako (1971) who found a lower plasma sodium than normal in a hypertensive population of 31 patients. His values were  $131 \pm 6$  mmol/l and  $3.4 \pm 0.66$  mmol/l for sodium and potassium respectively.

Salako's finding of a lower plasma potassium in hypertensives (as compared with the normal population) is confirmed. The difference observed here is 0.3 mmol/l. This difference is not statistically significant.

The cause of a higher plasma sodium in the hypertensives is not known, neither is it known whether the increase observed in the plasma (or for that matter in the red cells) is associated with a true increase in the total body sodium. None of the



TABLE XXXI

A. COMPARISON OF PLASMA SODIUM AND POTASSIUM  
OF NORMAL AND HYPERTENSIVE NIGERIANS

Subject	Plasma Na mmol/l	Plasma K mmol/l
Controls	128 $\pm$ 0.50 [95 - 155; 209]	4.6 $\pm$ 0.08 [2.5 - 5.5; 200]
Hypertensives	138 $\pm$ 0.64 [129 - 145; 39]	3.7 $\pm$ 0.08 [3.0 - 4.8; 35]
Difference	10 $\pm$ 0.81	0.9 $\pm$ 0.11
t	12.35	8.18
p	< 0.001	< 0.001

B. COMPARISON OF PLASMA SODIUM AND POTASSIUM  
OF THIS WORK WITH THOSE OF SALAKO 1971

Subject	Plasma Na mmol/l	Plasma K mmol/l
Hypertensives in this work	138 $\pm$ 0.64 [129 - 145; 39]	3.7 $\pm$ 0.08 [3.0 - 4.8; 35]
Hypertensives of Salako (1971)	131 $\pm$ 6 [119 - 140; 31]	3.4 $\pm$ 0.66 [2.2 - 4.9; 31]
Difference	7 $\pm$ 6.03	0.3 $\pm$ 0.66
t	1.16	0.45
p	> 0.1	> 0.5

patients was suffering from fluid retention from any cause at the time of the study.

The cause of the low plasma potassium is also uncertain, and it is not known whether this is associated with a true total body potassium deficit. By contrast, the RBC potassium was increased. In an attempt to explain the low plasma potassium, Salako (1971) suggested that it is possible that the low potassium level observed in many patients is related to renal dysfunction either as a cause or as an early manifestation. All the patients in the group had normal blood urea and plasma creatinine (Table xxii) and none was known to have hyposthenuria. Creatinine clearance was not done in any of the patients, so one cannot exclude early renal decompensation as Akinkugbe (1972) has pointed out that renal failure occurs more commonly in this community than in European communities in the course of essential hypertension.

TABLE XXXII

COMPARISON OF  $\frac{Na_o}{Na_i}$  AND  $\frac{K_o}{K_i}$  IN 31 CONTROL

SUBJECTS AND 30 HYPERTENSIVES

Subject	$\frac{Na_o}{Na_i}$	$\frac{K_o}{K_i}$
1. Controls	$15.6 \pm 1.24$ $[6.5 - 31.8; 30]$	$0.044 \pm 0.001$ $[0.03 - 0.058; 30]$
2. Hypertensives	$12.2 \pm 0.94$ $[4.5 - 25.0; 30]$	$0.042 \pm 0.001$ $[0.030 - 0.053]$
1 vs 2 difference	$3.4 \pm 1.56$ $t = 2.18$ $P > 0.01$	$0.002 \pm 0.001$ $t = 2.00$ $P > 0.01$

Comparison of intra-cellular sodium and potassium with plasma sodium and potassium in the normotensive and hypertensive groups. (Table XXXII)

In figures 1 and 1, the intracellular sodium ( $Na_i$ ) and potassium ( $K_i$ ) have been plotted against the plasma sodium ( $Na_o$ ) and potassium ( $K_o$ ) of the normotensive and hypertensive groups. From the graphs, it is obvious that  $Na_i$  and  $K_i$  are independent of  $Na_o$  and  $K_o$  in both the normotensive and hypertensive groups.

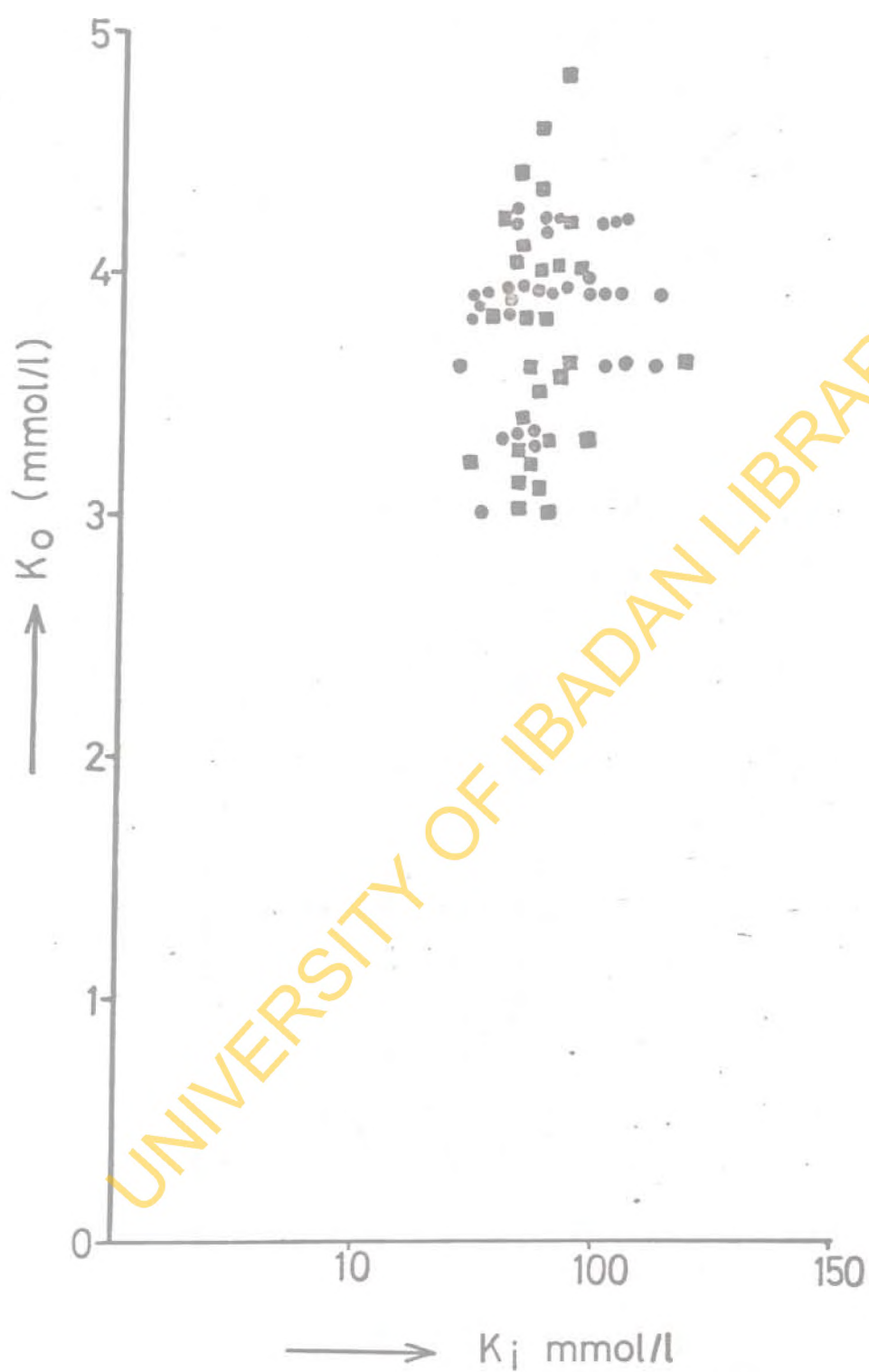
The mean ratio of  $Na_o$  to  $Na_i$  is higher in normotensives than in hypertensives by 3.4, the difference is statistically significant. The ratio of  $K_o$  to  $K_i$  is also higher in normotensives by 0.02, but the difference is not statistically significant (Table XXXI)

Passive Potassium Fluxes. (Tables XIV, XV, XVI and XVII; Figs. 10a & b and 11a & b)

Passive potassium efflux was about 7 times as fast in normotensives as in hypertensives during the first 10 minutes of the efflux studies. The K efflux rate in normotensives was significantly higher ( $P < 0.001$ ) than that in hypertensives at all intervals measured. This faster rate of passive



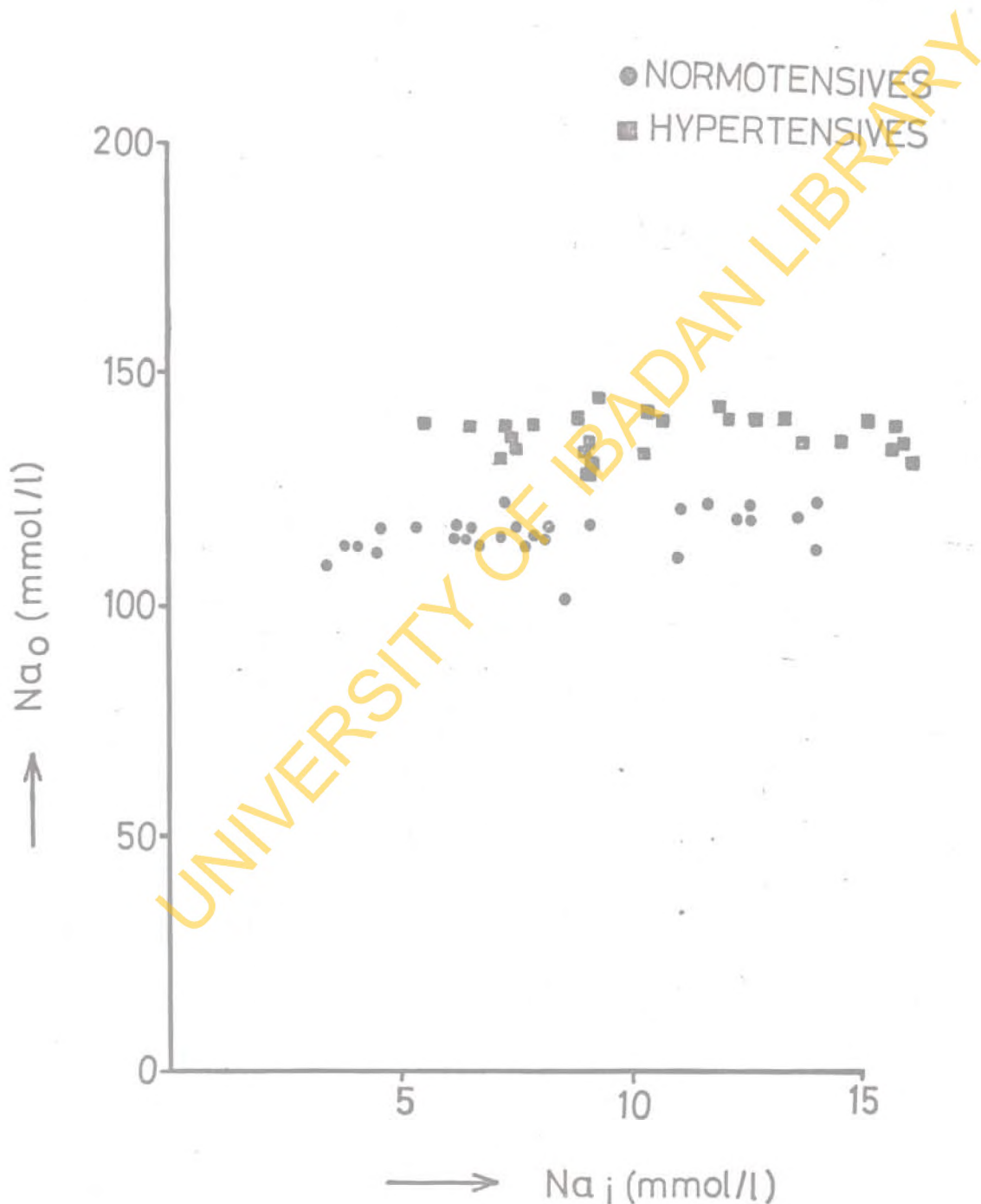
FIG. 18



THE RELATIONSHIP BETWEEN INTRACELLULAR  $K^+$  AND PLASMA  $K^+$  IN NORMOTENSIVE CONTROLS (●) AND HYPERTENSIVE SUBJECTS (■).

FIG. 19

THE RELATIONSHIP BETWEEN INTRACELLULAR  $\text{Na}^+$  AND PLASMA  $\text{Na}^+$  IN NORMOTENSIVE CONTROLS (●) AND AND HYPERTENSIVE SUBJECTS (■)



potassium efflux observed in normotensives probably accounts for the lower intracellular potassium observed in the normotensive controls (Table III).

3. In the normotensive group, the individual plots of potassium efflux rate with time overlapped in individuals with AA, AS and AC genotypes, suggesting that values for AS and AC are well within the limits covered by the AA subjects. A proper 't' test could not be done, as the numbers involved were too few.

4. In the hypertensive group, the one individual with AS genotype also had passive potassium fluxes which fitted within the limits of the AA genotype, suggesting that the results for the AS genotype are identical with those of the AA genotype.

Passive potassium efflux was fastest in the first 10 minutes in both the normotensive and hypertensive groups. This was because the incubating medium was completely devoid of potassium, hence a rapid efflux of potassium occurred down its concentration gradient. As the  $K_o$  gradually built up, between the 20th and 40th minute the efflux rate slowed considerably and the rate at the 40th minute was only  $\frac{1}{4}$  of the initial efflux rate. Further

slowing occurred but passive efflux was still recorded even at the end of the 4th hour in controls but by the end of the 3rd hour in most hypertensives, a steady state had been reached and the efflux rate at the end of the third hour was the same as the efflux rate at the end of the 4th hour. Observations were not made beyond the 4th hour.

#### Active Sodium Efflux

Normotensive Controls: Active sodium efflux for the normotensive controls was  $5.7 \pm 0.53$  mmol/l/hr. This value is significantly higher than the value reported by Smith and Samuel (1970) for 10 caucasian controls. The result obtained by Smith and Samuel was  $2.07 \pm 0.184$ . However, Smith and Samuel (1970) used a much more sophisticated technique with  $^{22}\text{Na}_a$ . Hence their standards of accuracy were much higher than mine. Tosteson (1955) also estimated active sodium efflux as 2.8 mmol/l/hr. in normal subjects, but he also used a radioactive technique.

Kurantsin-Mills et al. (1974) using a similar technique obtained mean values of  $6.48 \pm 3.2$  and  $6.70 \pm 4.7$  mmol/l/hr. in their normal control subjects. Their result was 0.78 - 1 mmol/l/hr.



higher than mine but the difference is not statistically significant. My apparently lower result is probably due to the fact that for my active transport studies, I only obtained results for pumps Ia and Ib, not for pump II. This is because Pump II was not blocked as ethacrynic acid was not used in my experimental set up. The mean active sodium efflux is also in close agreement with the results of Post and Jolly (1957) who used a similar method.

The sodium efflux results obtained for individuals with Hb genotypes AA, AS and AC did not show any statistically significant difference.

The efflux rate constant for my control subjects was  $0.19 \pm 0.02/\text{hr}$ . This value is justifiably lower than that of Smith and Samuel (1970) who reported  $2.07 \pm 0.184/\text{hr}$ ., since the red cell sodium for the subjects in this study is higher.

Hypertensive Subjects: (Pre-treatment) Active sodium efflux for hypertensive subjects was  $4.23 \pm 0.32 \text{ mmol/l/hr}$ . There is no significant difference between the sodium efflux rates for

normotensive controls and hypertensive subjects. The efflux rate constant in hypertensives is significantly lower than in normotensives, probably because the intracellular sodium in hypertensive subjects is higher.

Available literature on active sodium transport in hypertension is very scanty. Edmondson, et al. (1975) also reported a significantly lower sodium efflux rate constant in patients with essential hypertension.

Hypertensive subjects - (on treatment for 1 year) - RBC sodium and potassium content and transport were again studied in 7 subjects (Subjects 1 - 7, appendix), 1 year from the start of treatment. The results obtained (summarised in Table XVII) showed that treatment did not alter the findings discussed above.

Passive Sodium Influx and Efflux - passive sodium influx or efflux rate was not estimated, but it was conclusively shown (Table XIV) that the rate of passive <sup>entry</sup> of sodium into RBC of hypertensive subject is much higher than that of normal subjects.

These results demonstrate an abnormality in the red cell sodium, potassium and water content of hypertensive subjects. An abnormality in the active and passive sodium transport is also present as shown by the faster rate of passive sodium influx and the lower rate constant for sodium influx. An abnormality in the passive potassium efflux has also been demonstrated. The reasons for these abnormalities are not known.

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TABLE XXXIII

COMPARISON OF RBC SODIUM CONTENT IN 64 HYPERTENSIVE  
 SUBJECTS WITH DIFFERENT HB. GENOTYPES (AA, AS, AC)

Subjects	Na mmol/l	Difference	t
1. AA genotype	13.7 $\pm$ 0.91 (3.6-24.5; 45)	1 vs 2 3.2 $\pm$ 1.94	1.65 $p > 0.1$
2. AS genotype	16.9 $\pm$ 1.71 (9.0-31.3; 18)	1 vs 3 ) 2 vs 3 )	cannot be compared as N in 3 = 1
3. AC genotype	17.5 (-----; 1)		

In parentheses: range, number of subjects.

N = number of subjects.



CHAPTER VII

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CHAPTER 7

CONCLUSION

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In the study reported in this thesis, the sodium and potassium content of erythrocytes in healthy Nigerians were determined in order to obtain normal values for the population.

The normal values obtained were compared with previous results in Africans and with the results obtained in caucasians living in Europe and caucasians living in Nigeria.

From these comparisons, it was shown that the RBC sodium in Africans is significantly higher than that in caucasians, while the RBC potassium and water are similar in both groups of subjects.

The difference obtained between the values for Nigerians and Europeans was considered to be due to racial rather than environmental factors because a similar difference was found in the values for Nigerians and Europeans living in Nigeria.

Comparable results were obtained for RBC sodium, potassium and water content in healthy Ghanaians and Nigerians, when the results obtained in both groups are expressed in identical units of measurement.

It is however considered that the large numbers studied in this study may need to be repeated in other African groups for a more valid comparison of normal values.

Passive potassium efflux was studied in healthy controls and the results showed a passive efflux rate which progressively diminished until a steady state was reached after about 3 hours. Active potassium transport was not studied because the technique used was not considered sensitive enough for this measurement.

Active and passive sodium transport across the erythrocyte membrane was measured. The results showed that in healthy subjects the net active efflux was higher than those reported in caucasians. This result would suggest that the high sodium content of red cells obtained in the Nigerians studied relative to caucasians is not due to a low activity of the sodium pump in Nigerians. A study of the other factors which may lead to a high sodium content in Nigerians would be an interesting follow-up to this study.



The healthy controls used in this study were analysed for differences in haemoglobin genotypes. Subjects with Hb genotypes AA, AS and AC were identified. No significant difference was found in the sodium and potassium content and transport in the erythrocytes with these various Hb genotypes.

The hypertensive subjects studied showed higher intra-erythrocyte sodium and potassium than healthy controls. On the contrary the plasma sodium of these hypertensives was higher and the potassium lower than in healthy controls. The significance of these findings in hypertensive patients is not clear, but is of interest in view of the well-known abnormalities in the renin-angiotensin system in hypertensive blacks. This again would be a useful area to pursue in future studies.

The method used in this study was that of chemical analysis of sodium and potassium. This method is time consuming, and, especially in the transport studies where small changes are monitored, is relatively insensitive. It would

be interesting to repeat some of the experiments using the far more sensitive radio-active tracer technique. However, as a first large scale study of intracellular cations in healthy Nigerians and in hypertensives - both with different haemoglobin genotypes - it is hoped that this study will stimulate further interest in electrolyte metabolism in health and disease in the population.

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Subject No.	Age/Sex	Genotype	Symptom	Supine B.P.	Fundus	X-ray Heart size	ECG Heart size
1	52 M	AA	Nil	185/110	Grade I	Normal	L.V.
2	50 F	AA	Nil	160/110	" I	Normal	L.V.
3	50 M	AA	Nil	190/110	" I	Normal	L.V.
4	50 M	AS	old stroke	160/110	Not Examined	enlarged	L.V.
5	60 F	AA	Nil	170/100	-	Normal	L.V.
6	40 F	AA	Palpitations	160/100	-	"	Norm
7	40 M	AA	Headache	210/125	-	"	Norm
8	38 M	AA	Heavy head	180/120	II	"	Norm
9	40 M	AA	Nil	150/100	-	"	Norm
10	50 F	AS	Nil	155/100	-	"	Norm
11	54 F	AA	Nil	155/100	-	"	Norm
12	59 M	AS	Nil	165/115	-	"	Norm
13	43 M	-	Nil	220/130	Normal	"	Norm
14	38 M	AA	Nil	180/130	Normal	"	Norm
15	41 F	AS	Nil	140/100	Normal	Normal	Norm
16	23 F	AA	Nil	155/100	I	Large	L.V.
17	40 M	AA	Nil	210/125	I	Normal	Norm
18	37 M	AA	Chest pain	160/110	Normal	Normal	L.V.
19	33 F	AA	Nil	180/110	Nil	Large	L.V.
20	41 F	AA	Nil	165/110	Normal	Normal	L.V.
21	48 F	AA	Nil	200/115	Normal	Normal	Norm
22	50 F	AA	Nil	150/100	I	Large	Large
23	50 F	AA	Nil	230/130	I	Normal	L.V.
24	60 M	AS	Nil	200/130	Cataracts	Large	L.V.

Subject No.	Age/Sex	Genotype	Symptom	Supine B.P.	Fundus	X-ray Heart size	ECG Heart size
25	51 M	AA	Nil	130/90	I	Normal	L.V.
26	51 M	-	Nil	160/120	Normal	Normal	Norm
27	50 M	AA	Dyspnoea	220/160	II	Normal	L.V.
28	51 F	AS	Nil	140/100	I	Normal	Norm
29	30 M	AA	Nil	170/120	III	Large	L.V.
30	27 F	AS	Nil	140/80	I	Normal	Norm
31	60 F	-	Nil	145/90	I	Normal	L.V.
32	24 M	AS	Nil	180/110	Normal	Nil	Nil
33	60 M	AA	Nil	220/130		Normal	L.V.
34	60 F	AA	Nil	180/105	Cataracts Not Examined	Large	L.C.
35	35 M	AA	Nil	140/100		Normal	Norm
36	60 F	AS	Nil	200/120		Large	L.V.
37	62 F	-	Nil	160/90	Normal	Large	L.V.
38	50 F	AA	Nil	180/120	II	Large	L.V.
39	48 M	AA	Nil	150/100	Not seen	Normal	Norm
40	60 M	AS	Nil	205/110	Normal	Large	L.V.
41	45 F	AA	Nil	150/100		Normal	Norm
42	42 F	AA	Nil	215/145		Large	Large
43	50 M	AA	Nil	190/120	II	Large	L.V.
44	47 F	AA	Nil	200/115	III	Large	L.V.
45	53 F	AS	Nil	285/100	II	Large	L.V.
46	48 F	AS	Nil	230/150	II	Large	L.V.
47	40 F	AA	Nil	210/120	II	Large	L.V.



Subject No.	Heart Size Clinical Exam	Blood Urea mg%	I.V.P.	Serum Creatinine mg%	Proteinuria in M.S.U.
1	Normal	22	Normal	1.0	Nil
2	Normal	29	"	1.0	Nil
3	Normal	33	"	1.6	Nil
4	Normal	24	"	1.4	Nil
5	Normal	36	"	1.4	Nil
6	Normal	20	"	1.0	Nil
7	Normal	29	"	1.2	++
8	Normal	24	"	0.8	Nil
9	"	20	"	1.0	Nil
10	"	29	"	-	Nil
11	"	23	"	-	Nil
12	"	24	"	1.0	Nil
13	"	21	"	1.4	Nil
14	"	19	"	1.0	Nil
15	"	19	"	2.0	Nil
16	"	22	"	0.8	Nil
17	"	26	"	1.4	Nil
18	"	26	"	1.9	Nil
19	"	20	"	2.0	+
20	"	29	"	1.3	Nil
21	"	25	"	1.0	Nil
22	"	25	Not done	2.0	Nil
23	"	36	"	1.3	Nil
24	"	20	"	1.0	Nil

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Subject No	Heart Size Clinical Exam	Blood Urea MG%	I.V.P.	Serum Creatinine mg%	Proteinuria in M.S.U.
25	Normal	24	Not done	1.2	Nil
26	"	28	"	1.1	Nil
27	Cardiomegaly	36	"	1.2	Nil
28	Normal	22	Normal	1.2	Nil
29	Large	42	Not done	1.1	++
30	Normal	18	Chronic Pyelone- phritis	1.3	Nil
31	Normal	24	Not done	1.4	+
32	Normal	28	Normal	2.3	Nil
33	Normal	41	Not done	1.8	Nil
34	"	27	"	-	Nil
35	"	47	Normal	0.9	+++
36	"	20	Not done	0.6	Nil
37	"	24	"	1.0	Nil
38	"	24	"	-	Nil
39	Normal	26	Normal	2.3	Nil
40	"	26	Normal	2.1	Nil
41	"	28	Not done	1.5	Nil
42	Large	30	"	2.4	++
43	Large	19	"	1.5	Nil
44	Normal	28	Normal	-	Nil
45	Normal	24	Normal	1.4	Nil
46	Normal	20	Normal	1.3	Nil
47	Normal	32	Chronic Pyelone- phritis	2.0	trace

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