MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A
**Evaluation of Erythrocyte Sodium-22 Influx as a Laboratory Test for the Diagnosis of Essential Hypertension**

**PERFORMING ORGANIZATION NAME AND ADDRESS**
AFIT STUDENT AT: University of Nebraska

**REPORT DATE**
Dec 82

**NUMBER OF PAGES**
56

**DECLASSIFICATION/RECLASSIFICATION SCHEDULE**
APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED

**SUPPLEMENTARY NOTES**
APPROVED FOR PUBLIC RELEASE: IAW AFR 190-17

**KEY WORDS**
AFIT, Wright-Patterson AFB, OH

**ABSTRACT**
ATTACHED
ABSTRACT

A simple new procedure to measure erythrocyte uptake of sodium-22 was evaluated as a laboratory test for the diagnosis of essential hypertension. Erythrocytes from 25 normotensive controls, 18 normotensive offspring of essential hypertensives, 25 patients with essential hypertension and 25 patients with hypertension secondary to renal disease, were incubated in an isotonic buffer containing sodium-22. Intracellular sodium and potassium were also measured to determine the influence of these cations on net sodium influx.

Mean sodium-22 influx was significantly higher in patients with essential hypertension (0.278 ±0.079 mmol/liter cells/hour) than normotensive controls (0.216 ±0.037) or secondary hypertensives (0.220 ±0.047)(p<0.01). Erythrocyte sodium and potassium concentration in patients with essential hypertension is not significantly different from controls. Sodium influx does not appear to be affected by the intracellular concentration of Na⁺ or K⁺. The correlation coefficient between these parameters was -0.17.

Overlap occured in all four groups. Fourteen of 25 essential hypertensives had results within 2 SD of the mean of the control group, suggesting that essential hypertension may have multiple etiologies. However, all patients with secondary hypertension had normal results, indicating that alterations in membrane sodium transport are not acquired simply as a consequence of high blood pressure. Significant age, sex or drug related influences were not observed. We conclude that this procedure may provide important diagnostic and prognostic information for the treatment of patients with essential hypertension.

KEY REFERENCES


AFIT/CI/NR 82-64T

AFIT RESEARCH ASSESSMENT

The purpose of this questionnaire is to ascertain the value and/or contribution of research accomplished by students or faculty of the Air Force Institute of Technology (ATC). It would be greatly appreciated if you would complete the following questionnaire and return it to:

AFIT/NR
Wright-Patterson AFB OH 45433

RESEARCH TITLE: Evaluation of Erythrocyte Sodium-22 Influx as a Laboratory Test for the Diagnosis of Essential Hypertension

AUTHOR: James Douglas Sargent

RESEARCH ASSESSMENT QUESTIONS:

1. Did this research contribute to a current Air Force project?
   ( ) a. YES  ( ) b. NO

2. Do you believe this research topic is significant enough that it would have been researched (or contracted) by your organization or another agency if AFIT had not?
   ( ) a. YES  ( ) b. NO

3. The benefits of AFIT research can often be expressed by the equivalent value that your agency achieved/received by virtue of AFIT performing the research. Can you estimate what this research would have cost if it had been accomplished under contract or if it had been done in-house in terms of manpower and/or dollars?
   ( ) a. MAN-YEARS  ( ) b. $_______

4. Often it is not possible to attach equivalent dollar values to research, although the results of the research may, in fact, be important. Whether or not you were able to establish an equivalent value for this research (3. above), what is your estimate of its significance?
   ( ) a. HIGHLY SIGNIFICANT  ( ) b. SIGNIFICANT  ( ) c. SLIGHTLY SIGNIFICANT  ( ) d. OF NO SIGNIFICANCE

5. AFIT welcomes any further comments you may have on the above questions, or any additional details concerning the current application, future potential, or other value of this research. Please use the bottom part of this questionnaire for your statement(s).

NAME
GRADE
POSITION

ORGANIZATION
LOCATION

STATEMENT(s):
EVALUATION OF ERYTHROCYTE SODIUM-22 INFUX
AS A LABORATORY TEST FOR THE
DIAGNOSIS OF ESSENTIAL HYPERTENSION

James Douglas Sargent, Capt, USAF, BSC

A Thesis, 56 pages

Master of Science Degree

University of Nebraska Medical Center

1982
ABSTRACT

A simple new procedure to measure erythrocyte uptake of sodium-22 was evaluated as a laboratory test for the diagnosis of essential hypertension. Erythrocytes from 25 normotensive controls, 18 normotensive offspring of essential hypertensives, 25 patients with essential hypertension and 25 patients with hypertension secondary to renal disease, were incubated in an isotonic buffer containing sodium-22. Intracellular sodium and potassium were also measured to determine the influence of these cations on net sodium influx.

Mean sodium-22 influx was significantly higher in patients with essential hypertension (0.278 ±0.079 mmol/liter cells/hour) than normotensive controls (0.216 ±0.037) or secondary hypertensives (0.220 ±0.047)(p<0.01). Erythrocyte sodium and potassium concentration in patients with essential hypertension is not significantly different from controls. Sodium influx does not appear to be affected by the intracellular concentration of Na⁺ or K⁺. The correlation coefficient between these parameters was -0.17.

Overlap occurred in all four groups. Fourteen of 25 essential hypertensives had results within 2 SD of the mean of the control group, suggesting that essential hypertension may have multiple etiologies. However, all patients with secondary hypertension had normal results, indicating that alterations in membrane sodium transport are not acquired simply as a consequence of high blood pressure. Significant age, sex or drug related influences were not observed. We conclude that this procedure may provide important diagnostic and prognostic information for the treatment of patients with essential hypertension.

KEY REFERENCES


TABLE OF CONTENTS

I. INTRODUCTION ..................................................... 1

II. LITERATURE REVIEW .................................................. 3
   A. PHYSIOLOGY OF BLOOD PRESSURE CONTROL ......................... 3
      1. NEURAL ..................................................... 3
      2. HUMORAL .................................................... 3
      3. RENAL ....................................................... 4
   B. HYPERTENSION ..................................................... 5
      1. DEFINITION .................................................. 5
      2. CLASSIFICATION .............................................. 5
      3. PREVALENCE ................................................ 7
      4. RISK FACTORS ............................................... 7
         a. FAMILY HISTORY .......................................... 7
         b. SODIUM INTAKE ............................................ 7
         c. CALCIUM INTAKE .......................................... 8
         d. BODY WEIGHT ............................................ 8
         e. OTHER FACTORS .......................................... 9
   C. ERYTHROCYTE SODIUM TRANSPORT ................................. 9
      1. MECHANISMS .................................................. 9
         a. SODIUM-POTASSIUM PUMP ................................ 9
         b. SODIUM-POTASSIUM COTRANSPORT ......................... 11
         c. LITHIUM - SODIUM COUNTERTRANSPORT .................... 11
         d. SODIUM - CALCIUM EXCHANGE .............................. 12
      2. THEORETICAL IMPLICATIONS IN ESSENTIAL HYPERTENSION ....... 12
   D. PREVIOUS STUDIES ............................................... 15

III. MATERIALS AND METHODS .......................................... 19
   A. SODIUM-22 ($^{22}\text{Na}^+$) UPTAKE STUDY ...................... 19
### TABLE OF FIGURES

| FIGURE 1 | MECHANISMS OF SODIUM TRANSPORT IN ERYTHROCYTES |
| FIGURE 2 | LINEARITY OF THE $^{22}\text{Na}$ INFLUX PROCEDURE |
| FIGURE 3 | MEAN VALUES AND RANGES OF SODIUM-22 INFLUX IN FOUR GROUPS OF SUBJECTS |
| FIGURE 4 | SODIUM-22 INFLUX IN FOUR GROUPS OF SUBJECTS, SUBCLASSIFIED BY SEX |
| FIGURE 5 | COMPARISON OF MEAN MALE AND FEMALE SODIUM-22 INFLUX VALUES |
| FIGURE 6 | COMPARISON OF MEAN INTRACELLULAR SODIUM CONCENTRATIONS FOR THE FOUR GROUPS STUDIED |
| FIGURE 7 | COMPARISON OF INTRACELLULAR SODIUM CONCENTRATIONS, SUBCLASSIFIED BY SEX |
| FIGURE 8 | COMPARISON OF MEAN INTRACELLULAR POTASSIUM CONCENTRATIONS FOR THE FOUR GROUPS STUDIED |
TABLE OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>TABLE 1</td>
<td>ETIOLOGIC CLASSIFICATION OF HYPERTENSION</td>
</tr>
<tr>
<td>TABLE 2</td>
<td>INDIVIDUAL VARIATION IN SODIUM-22 INFLUX</td>
</tr>
<tr>
<td>TABLE 3</td>
<td>SUMMARY OF RESULTS: SODIUM-22 INFLUX AND INTRACELLULAR SODIUM AND POTASSIUM IN THE FOUR GROUPS STUDIED</td>
</tr>
</tbody>
</table>
I. INTRODUCTION

The pathogenesis of human essential hypertension is complex and poorly understood. A combination of genetic and environmental influences appears to be involved, with sodium metabolism occupying a central position.

The study of sodium transport across cell membranes provides a good approach to investigating the mechanisms involved in essential hypertension. Sodium is transported by several different pathways, involving both active and passive processes, which help to maintain a stable concentration of sodium in the cell.

Sodium transport is altered in erythrocytes of many patients with essential hypertension and in some of their first degree offspring. However, the transport defects are absent in patients with secondary hypertension, suggesting that these defects are not acquired simply as a consequence of high blood pressure. Changes in erythrocyte sodium and potassium absolute levels have also been reported by some investigators. The molecular basis for these defects has not been determined but tests devised to measure membrane sodium transport may provide valuable diagnostic information.

Mahoney et al.29 have recently reported a rapid, simple and reproducible test for the estimation of red cell Na⁺ transport. The objective of the study reported here was the evaluation of red cell Na⁺ transport as a possible diagnostic tool in the clinical laboratory, including confirmation of
originally reported results, assessment of the method's sensitivity and specificity, identification of possible sources of error and application to a clinical setting. Intracellular sodium and potassium were also measured to determine if these parameters influence the total sodium influx, as suggested by Henningsen and Nelson. 25
II. LITERATURE REVIEW

A. Physiology of Blood Pressure Control

1. Neural

Arterial blood pressure is a function of cardiac output and peripheral vessel resistance. Both functions are controlled by the autonomic nervous system. Receptors located in the walls of large arteries respond to pressure or volume stimuli and are able to modify the vasoconstrictive effects of the vasomotor center. A fall in circulatory pressure stimulates increased cardiac output and constriction of peripheral arterioles, thereby increasing blood pressure.\(^5\)

Sympathetic nerve transmission is mediated by catecholamines. Epinephrine and norepinephrine are synthesized in sympathetic nerve cells and the adrenal medulla. The arrival of a nerve impulse at a sympathetic nerve ending triggers catecholamine discharge. Entry of calcium ions into the cell appears to be necessary for the release process.\(^5\) Catecholamine released from the nerve cell acts at appropriate receptor sites.

2. Humoral

A complex interplay of humoral pressor and depressor substances serves to modulate vascular resistance and intravascular fluid volume.

Renin is a proteolytic enzyme synthesized and stored in the juxtaglomerular apparatus of the kidney. It acts upon its substrate angiotensinogen to produce angiotensin I.
Hydrolysis by a converting enzyme produces angiotensin II, one of the most powerful vasoconstricting substances known. Angiotensin II acts directly on the CNS to increase peripheral vascular resistance and stimulates release of the sodium-retaining hormone aldosterone. Both actions increase arterial blood pressure. Release of renin by the kidney is directly affected by angiotensin II concentration, renal blood flow, and sodium concentration in the distal tubules. Sympathetic alpha and beta-adrenergic receptors are also involved in stimulation of renin secretion.

Kallikrein is a plasma enzyme whose action is similar to renin. It acts on the substrate kininogen to release the active peptide bradykinin. Bradykinin stimulates arterial vasodilation and renal sodium and water excretion, thereby decreasing blood pressure. The kininase which inactivates bradykinin is the same enzyme that converts angiotensin I to angiotensin II.

Angiotensin and bradykinin stimulate synthesis and release of renal prostaglandins. These compounds exhibit both vasoconstrictive and vasodilatory effects and help to modulate renin secretion and aldosterone action.

3. Renal

Neural and humoral systems appear to be designed for short-term control of blood pressure. Long-term control is achieved by the regulation of fluid volume by the kidney. The kidneys regulate circulatory volume by excreting sodium and water when arterial pressure is high and retaining sodium and water when
pressure is low. Urine output is adjusted to equal net fluid intake, thereby maintaining arterial pressure at its normal level.

B. Hypertension

1. Definition

Hypertension is the result of a perturbation in one or more of the control systems affecting blood pressure. High blood pressure is not a disease in itself but plays a significant role in the etiology of many diseases. No sharp boundary exists between normal and abnormal blood pressure, however generally accepted reference ranges have been established. Hypertension is defined in the adult as a blood pressure of 160 mmHg or greater systolic and 95 mmHg or greater diastolic. Normotension is defined as a blood pressure of 139 mmHg or less systolic, and 89 mmHg or less diastolic. Values of 140-159 mmHg systolic and 90-94 mmHg diastolic are considered borderline.

2. Classification

Hypertension is termed primary when the cause is unknown and secondary when a specific cause can be determined (Table 1). The term "essential hypertension" is often used to mean primary hypertension. Approximately 90% of hypertensive patients are classified as primary. About 5% of patients are hypertensive due to chronic renal disease. Less than 5% of hypertension cases are surgically curable. These include renovascular hypertension, primary aldosteronism and pheochromocytoma.
TABLE 1
ETIOLOGIC CLASSIFICATION OF HYPERTENSION

Primary, "essential"
- Labile (borderline)
- Benign
- Malignant

Secondary
- Aortic coarctation
- Renal
  - Parenchymal
  - Renovascular
- Endocrine
  - Primary aldosteronism
  - Pheochromocytoma
  - Congenital adrenal hyperplasia
    - 11-Hydroxylase
    - 17-Hydroxylase
  - Cushing's syndrome
  - Liddle's syndrome
  - Deoxycorticosterone induced
  - 18-Hydroxycorticosterone induced
  - Hyperthyroidism
  - Primary reninism

Drug Induced
- Oral contraceptives
- Steroids
- Licorice and derivatives
- Carbenoxalane
- Sodium bicarbonate
3. **Prevalence**

Hypertension is a common health problem, especially in industrialized countries. The currently accepted figure for prevalence in the United States is 18% of the adult population. The prevalence in children and adolescents is much lower than adults: approximately 2%. 

4. **Risk Factors**

   a. **Family History**

   Prevalence of hypertension in individuals with a positive family history for hypertension is almost twice as high as those with a negative history. This association between increased risk and a positive family history would indicate a genetic influence. However, not all individuals with a family history will develop high blood pressure. Environmental factors, most notably diet, may prevent development of hypertension despite a genetic predisposition.

   b. **Sodium Intake**

   Habitual high salt intake is widely held to be important in the genesis of hypertension. Humans ingest much more sodium than needed to maintain homeostatic mechanisms. It is unlikely, however, that a high sodium intake raises the blood pressure of most people. Many studies have failed to show a correlation between sodium intake and blood pressure. Alternatively, it is possible that only a susceptible minority within a population will experience an increased blood pressure when exposed to a high sodium intake. A threshold relationship has been proposed in support of this concept. Intake of sodium
above a certain level in sensitive individuals would predispose them to development of hypertension.\textsuperscript{39}

c. Calcium Intake

Recently, alterations in calcium intake and metabolism have been reported in hypertensive individuals. These disturbances include chronic depression of serum ionized calcium, altered membrane binding, low dietary intake and elevated urinary excretion.\textsuperscript{30,31,32} Vascular smooth muscle and adrenergic nerve cell activity are dependent on calcium fluxes across the plasma membrane.\textsuperscript{3} A reduction in dietary calcium intake may exacerbate other existing abnormalities associated with hypertension.

d. Body Weight

A high correlation exists between relative body weight and blood pressure. This relationship exists in all age-sex-race groups studied, for persons both with and without a positive family history of hypertension.\textsuperscript{39} Obesity is a risk factor for development and progression of hypertension, and hypertensives tend to become obese. The two conditions may be genetically linked.\textsuperscript{37}

The mechanism of this risk factor remains unclear. De Luise et al. found reduced ion-transport activity of the sodium-potassium pump and increased sodium concentration in red cells of obese persons.\textsuperscript{13} Mir et al. also reported elevated red cell sodium levels as well as increased passive influx of sodium-22.\textsuperscript{34} Sims suggests that, since insulin can affect renal sodium re-
absorption and membrane sodium transport, the insulin resistance and hyperinsulinemia of the obese may directly contribute to hypertension in susceptible individuals.\(^\text{37}\)

e. **Other Factors**

There is evidence that hyperglycemia, hyperuricemia and hypercholesterolemia may be independently related to blood pressure and to prevalence of hypertension. Each of these traits is, in part, diet dependent.\(^\text{39}\)

C. Erythrocyte Sodium Transport

1. **Mechanisms**

The intracellular concentration of sodium (7 mmol/liter) and potassium (100 mmol/liter) is approximately the reverse of the relative concentrations of these ions in the extracellular fluid.\(^\text{41}\) Sodium ions are able to enter and potassium ions exit the cell via the passive permeability of the cell membrane. Cells maintain their concentration gradients by active and passive transport systems (Figure 1). The sodium and potassium gradients are involved in propagation of the nerve impulse, reabsorption of solutes by the kidney, uptake of nutrients in the intestine and other cellular functions.

a. **Sodium-Potassium Pump**

A specific enzyme in the cell membrane is the major system responsible for active transport of sodium and potassium. It utilizes energy from the hydrolysis of ATP and has been termed the sodium-potassium ATPase. This pump actively transports sodium out of and potassium into the cell. The ratio
FIGURE 1: MECHANISMS OF SODIUM TRANSPORT IN ERYTHROCYTES
of sodium to potassium transport is tightly coupled: three sodium ions exit for every two potassium ions that enter. Transport of either ion requires the presence of the other ion on the opposite side of the membrane. Binding to specific sites on the enzyme facilitates the transport process. Intracellular sodium is thought to be rate-limiting for enzyme activity in many cells. The pump is stimulated by addition of sodium ions to the intracellular fluid and inhibited by lowering the concentration of sodium. Cell sodium concentration normally remains at a very constant value, suggesting a tight linkage between the pump and intracellular sodium content. This relationship may be altered in certain disease states.

b. Sodium-Potassium Cotransport

Inhibition of the Na+,K+-pump with cardiac glycosides results in a net residual sodium transport against the concentration gradient. This observation revealed the existence of transport systems independent of the pump. The sodium-potassium cotransport catalyzes a simultaneous 1:1 influx or efflux of both Na+ and K+. The specific transport proteins involved have not been characterized but are thought to derive energy from the large, outwardly directed K+ gradient. The exact function of cotransport is also unclear but probably involves a "fine-tuning" of intracellular Na+ and K+, in cooperation with the pump.

c. Lithium - Sodium Countertransport

This system promotes the exchange of sodium for sodium, lithium for lithium, or lithium for sodium. The transport
process is tightly coupled, exchanging one intracellular cation for one extracellular cation. Such a system can perform exchange but no net transport of ions. The physiologic significance of the system is therefore obscure. Transport energy is derived from electrochemical ion gradients, as in cotransport. The maximum rate of countertransport varies considerably from person to person, at least in part because of genetic factors.

d. **Sodium – Calcium Exchange**

Calcium ions continuously penetrate cells due to the large concentration gradient. To prevent intracellular over-load, calcium ions (Ca²⁺) must be continuously pumped out of cells. Two systems have been identified for this purpose: a specific ATPase and a Na⁺/Ca²⁺ exchanger. Both systems coexist in the plasma membrane. The Na⁺/Ca²⁺ exchange seems to be particularly important in excitable cells such as smooth muscle, neurons and myocardium. The probable stoichiometry of Na⁺– Ca²⁺ exchange is one Ca²⁺ ion pumped out in exchange for three Na⁺ ions pumped in. Since a net transport of ions is achieved, Na⁺ ions appear to play a critical role in maintaining Ca²⁺ balance in many cells.

2. **Theoretical Implications in Essential Hypertension**

A strong correlation between sodium metabolism and hypertension is widely recognized. However, the underlying mechanisms which provide this correlation remain unclear. Cell sodium transport systems are being studied to support a basic hypothesis: that abnormal sodium transport across cell mem-
branes is involved in development of at least some forms of hypertension. 42

Red blood cells are often used to study sodium transport because they are readily available and easy to work with. However, a direct causal relationship between red-cell ion transport and hypertension seems unlikely. More plausibly, an observed transport disorder in erythrocytes is present in another cell type involved in blood pressure control. 36

Most forms of hypertension are associated with an increased peripheral resistance due to abnormal vascular smooth muscle tone. Calcium ions are the immediate trigger for contraction in smooth muscle. 3 Intracellular sodium controls calcium concentration via the Na⁺-Ca²⁺ exchange. Blaustein has proposed that an increase in cytoplasmic sodium could cause a concordant rise in intracellular calcium, leading to increased vascular tone. 3,36 Smooth muscle cells are very sensitive to changes in intracellular calcium. Therefore, even a small change in the Na⁺/Ca²⁺ balance could be manifested as a change in tension. 4

The same concept can be applied to cells of the adrenergic nervous system. Electrical stimulation leads to a large influx of Ca²⁺ ions, triggering release of norepinephrine. The sodium-dependent calcium exchange system is important in removing this influx once stimulation has ceased. 2 An increase in intracellular sodium could inhibit removal of calcium ions and result in increased release of neurotransmitter. In support of this concept, elevated plasma catecholamine concen-
trations are now reported in patients with essential hypertension.5

A major hypothesis to explain hypertension involves neurogenic hyperactivity as the trigger. Increased norepinephrine, acting on alpha-adrenergic receptors, would promote increased vascular smooth muscle activity and tone.4 Adrenergic mechanisms are also known to be involved in control of renin secretion.12 Increased renin release would raise blood pressure via the renin-angiotensin-aldosterone axis. Initially, the resulting hypertension is intermittent or "labile". Hypertension becomes permanent when renal pressure-natriuresis relationships are "reset" to maintain sodium balance.5

Other relationships between abnormal sodium transport and hypertension are possible. The key appears to be in the interrelationship of Na and Ca ions. Possible mechanisms for altering this ratio include: increased passive permeability to sodium, inhibition of the Na\(^+\)/K\(^+\) pump or a decrease in Na\(^+\)/K\(^+\) cotransport.36

Both environmental and genetic factors influence blood pressure. An important concept is that gene products coding for abnormal sodium transport proteins may render an individual "sensitive" to environmental forces. This would explain why hypertension tends to segregate within families but not all persons with a family history will later develop high blood pressure. A compelling aspect of studying sodium transport is the possibility of identifying those individuals who are at increased risk. A positive transport "marker" could allow
proper prophylactic measures to be taken in an otherwise sus-
ceptible person.\textsuperscript{21,44}

D. Previous Studies

Abnormal transport of sodium ions across membranes of var-
ious cells in patients with essential hypertension has been
reported by several investigators. The specific transport
pathway affected and the results obtained vary considerably.\textsuperscript{42}

Garay \textit{et al.}\textsuperscript{17,18,19} reported that in essential hyper-
tension the net $\text{Na}^+$/influx ratio is abnormally
low. The abnormal ratio was observed in erythrocytes treated
with ouabain, a specific inhibitor of the $\text{Na}^+/\text{K}^+$ pump, and
was interpreted as being a defect in the cotransport system.
Cotransport activity was two to three times lower in erythro-
cytes of essential hypertensives than in normotensive controls.
A normal cotransport was found in patients with secondary hyper-
tension, suggesting that the abnormality in primary hyperten-
sion is not the consequence of high blood pressure but perhaps
part of the etiology. Normotensives and secondary hyperten-
sives with a family history of hypertension also showed abnor-
mal cotransport.\textsuperscript{20,21,22} These findings support the concept
of genetic predisposition to high blood pressure.

Meyer \textit{et al.}\textsuperscript{33} found a reduced cotransport activity in 54%
of subjects with one hypertensive parent and 74% of those with
two hypertensive parents. A study of 14 families over two or
three generations also showed the erythrocyte cation abnormal-
ity in one or more members of each consecutive generation.
They concluded that the cation transport defect is characteristic of essential hypertension and transmitted by a dominant and autosomal mode.

In another study, Cusi et al. found that two offspring of the same hypertensive mother and different fathers had different cation transport alterations, in spite of the apparently identical environment where they grew up.

The sodium-lithium countertransport system has been extensively studied by Canessa et al. They found the maximum rate of this countertransport to be significantly increased in erythrocytes of essential hypertensives and some of their first degree relatives. Patients with secondary hypertension had normal results. Again a genetic mode of transmission is suggested. However, the relation of elevated countertransport to the pathogenesis of essential hypertension is obscure since this pathway does not result in net transport of Na ions.

Canali et al. also studied sodium-lithium countertransport and confirmed a significant difference between essential hypertensives and controls. However, the elevation in countertransport was only observed in hypertensive subjects having at least one hypertensive parent. They suggest that countertransport is not a consistent marker for essential hypertension but is associated with a family prevalence.

Ibsen et al. found a significant sex difference in patients with essential hypertension. Sodium-lithium countertransport values were much higher in male hypertensives than in male controls. However, no difference in countertransport
could be demonstrated between female hypertensives and controls. They also found no significant difference between male and female children with and without a family history of hypertension. They suggest that a genetic sex linkage may occur or endocrine factors may modulate sodium transport mechanisms.

Worely et al.\textsuperscript{45} studied countertransport in erythrocytes or normal and hypertensive pregnant women. Surprisingly, they found that countertransport was increased in both groups when compared to non-pregnant subjects. They deduced that physiologic changes can temporarily alter sodium-lithium countertransport and postulated the transport activity may be a function of erythropoiesis, which is increased during pregnancy.

Uptake of sodium-22 ($^{22}\text{Na}^+$) in erythrocytes of essential hypertensives was found to be increased by Mahoney et al.\textsuperscript{29} Normotensive controls and secondary hypertensives showed normal uptake. Approximately 50% of offspring of hypertensive parents also showed increased Na$^+$ permeability. The uptake of $^{22}\text{Na}^+$ by red cells probably reflects a combination of ouabain-insensitive transport processes, including cotransport and countertransport.

In a different experiment, Henningsen and Nelson\textsuperscript{25} found that $^{22}\text{Na}^+$ influx was significantly higher in red cells from male offspring of essential hypertensives as opposed to male controls. Males were also noted to have higher influx values than females in each group. These results support the findings of Ibsen et al.\textsuperscript{26} concerning sex-related differences in sodium transport.
Racial differences in hypertension-associated sodium transport have also been observed. Etkin et al. measured passive influx of $^{22}\text{Na}^+$ in erythrocytes of American blacks with essential hypertension and found a complete lack of the abnormal transport characteristic of white hypertension. They concluded that essential hypertension in blacks may have a different genetic basis or a different expression. Garay et al. found lower cotransport values in African blacks than in European white subjects. The difference existed in both normal and hypertensive individuals, suggesting that the activity of sodium transport systems may vary from one population to another. This observation may help to explain the well established differences in prevalence of hypertension between populations.
A. Sodium-22 $^{22}\text{Na}^+$ Uptake Study

1. Reagents and Equipment

Packard Scintillation Counter, Model 2409 (Packard Instrument Co. Downs Grove, IL 60515)

Shaking Water Bath (Precision Scientific Co. Chicago, IL 60617)

Seroagu II centrifuge (Clay-Adams Inc. Parsippany, NJ 07054)

Adams Autocrit microcentrifuge (Clay-Adams Inc. Parsippany, NJ 07054)

Vortex-Genie (Scientific Instruments Inc. Bohemia, NY)

12 x 75 mm plastic tubes with cap (Curtin-Matheson Scientific Co. Kansas City, MO 64141)

Potassium Chloride Buffer, pH 7.40 @4°C The following reagent grade chemicals were added to approximately 900 ml of distilled water:

- Potassium Chloride - 10.81 gm (145 mM)
- Sodium Chloride - 0.29 gm (5 mM)
- D-Glucose - 1.80 gm (10 mM)
- Tris base - 1.21 gm (10 mM)
- MOPS - 2.10 gm (10 mM)
- Ouabain - 0.07 gm (0.1 mM)

(MOPS and Ouabain were obtained from Sigma Chemicals Co., St. Louis, MO 63178)

The pH was adjusted to 7.26 @24°C by dropwise addition of 1 N HCl and brought to a final volume of 1000 ml with distilled water. This solution yielded a pH of 7.4 ±0.02 at 4°C. Osmolality was checked and found to be 300 ±5 mOsm/Kg. The solution was stored at 4°C for no longer than two weeks to avoid bacterial growth.
Magnesium-Sucrose Buffer, pH 7.40 @4°C

The following reagent grade chemicals were added to approximately 900 ml of distilled water:

- Magnesium Chloride (MgCl₂·6H₂O) - 15.24 gm (75 mM)
- Sucrose - 25.67 gm (75 mM)
- D-Glucose - 1.80 gm (10 mM)
- Tris base - 1.21 gm (10 mM)
- MOPS - 2.10 gm (10 mM)

The pH was adjusted to 7.26 at 24°C using 1 N HCl and brought to a final volume of 1000 ml with distilled water. This solution yielded a pH of 7.4 ± 0.02 @4°C. Osmolality was checked and found to be 300 ± 5 mOsm/Kg. The solution was stored at 4°C for up to two weeks.

Sodium-22 (²²Na) Buffer, pH 7.4 @37°C

10 ml of potassium chloride buffer was pipetted into a 17 x 100 mm plastic tube (Cat. #2059, Falcon Plastics, Oxnard, CA 93030). One drop of 1 M Tris base was added to yield a pH of 7.4 at 37°C. Enough ²²Na (New England Nuclear, Boston, MA 02118 Cat. #NEZ-081) was added to the buffer to produce 100,000-200,000 cpm per 0.5 ml, usually 10ul. (Isotope should be purchased in sufficient quantity to allow several standardized batches to be prepared at once.) Buffer was stored at 4°C for up to two weeks or frozen for future use.

Biofluor (Cat. #NEF-961 New England Nuclear, Boston, MA 02118)

Trichloroacetic acid, 10% was prepared by dissolving 10 gm reagent grade TCA in 100 ml distilled water.
2. Specimen Collection and Storage

Venipuncture was performed on consenting subjects and 5 ml of venous blood was drawn into lithium-heparin coated vacuum tubes. Specimens were centrifuged and plasma and buffy coat were discarded. Red blood cells were either processed at once or stored for up to five days at 4°C. Prior to analysis, stored cells were re-equilibrated by adding an equal volume of complete Hanks Solution (lx) (GIBCO Laboratories, Grand Island, NY 14072) and incubating for one hour at 37°C.

3. Test Procedure

Erythrocyte sodium-22 influx was determined using the method of Mahoney et al. 29

a. Wash erythrocytes at least three times by suspending in cold potassium chloride buffer, centrifuging for two minutes at 1000 g, and aspirating the supernatant. Aspirate any remaining buffy coat or fibrin clots.

b. Gently mix the washed cells and remove a capillary sample for microhematocrit determination.

c. Pipet 0.5 ml of cells into duplicate 12 x 75 mm plastic tubes.

d. Add 0.5 ml of 22Na-buffer to each tube. Mix contents by gentle agitation.

e. Incubate cell suspensions at 37°C for exactly 30 minutes in a shaking water bath.

f. Rapidly wash cells by suspending in cold magnesium-sucrose buffer, centrifuging for one minute at 1000 g and aspirating the supernatant. (An automatic pipet works well for this step.)
g. Gently mix the washed cells, being sure to include any drops of liquid adhering to the sides of the tube. Remove a capillary sample for microhematocrit determination.

h. Pipet 0.3 ml washed, packed cells into a 12 x 75 mm plastic tube containing 0.6 ml 10% TCA. Immediately vortex the tube vigorously.

i. Centrifuge all tubes for three minutes at 1000 g.

j. Carefully remove 0.3 ml of clear supernatant into a 20 ml glass scintillation vial. Add 10 ml Biofluor and mix well.

k. Count vials for two minutes in a liquid scintillation counter, adjusted to maximum counting efficiency for $^{22}$Na. A vial containing 0.5 ml $^{22}$Na-buffer and 10 ml Biofluor must be included with each batch to determine total counts in the incubation mixture.

4. Calculations

The apparent Na$^+$ influx of each tube is calculated as follows:

a. $1.0 \text{ ml (incubation volume)} - \text{RBC volume (Hct x 0.5 ml)}$
   $= \text{extracellular volume (usually 0.60-0.65 ml)}$

b. Extracellular volume $\times 5$ (mmol Na$^+$/liter)
   $= \text{extracellular Na}^+ (\mu\text{mol})$

c. $\frac{\text{Total Cpm } ^{22}\text{Na}^+ \text{ in incubation}}{\text{Extracellular Na}^+} = \frac{\text{cpm/\mumol Na}^+}{\text{cpm/ml RBC/hr}}$

d. $\frac{\text{cpm/0.3 ml TCA extract}}{\text{RBC Hct/100}} \times 20 = \frac{\text{cpm/ml RBC/hr}}{\text{mmol Na}^+/\text{liter RBC/hr}}$

e. $\frac{\text{cpm/ml RBC/hr}}{\text{cpm/\mumol Na}^+} = \text{mmol Na}^+/\text{liter RBC/hr}$
Duplicate results should not differ by more than 10%. In all cases, the highest value was taken as the measure of Na⁺ influx.

B. Intracellular Sodium and Potassium

1. Reagents and Equipment

Flame Photometer, Model 443 (Instrumentation Laboratories, Lexington, MA 02173)

Automatic Dilutor, Model 444 (Instrumentation Laboratories, Lexington, MA 02173)

Adams Autocrit microcentrifuge (Clay-Adams Inc., Parsippany, NJ 07054)

Serofuge II centrifuge (Clay-Adams Inc., Parsippany, NJ 07054)

12 x 75 mm plastic tubes

Magnesium Chloride, 112 mmol/liter was prepared by dissolving 22.77 gm reagent grade magnesium chloride in one liter of distilled water. Store at 4°C.

Saponin Dissolve 2 gm hemolysis grade saponin in 10 ml magnesium chloride solution. Store at 4°C.

Electrolyte Standard, 6.0 mmol/L Na⁺/ 90.0 mmol/L K⁺

Dissolve 0.6709 gm KCl and 0.0345 gm NaCl in 100 ml distilled water. Store at room temperature.

2. Specimen Collection and Storage

Venous blood was collected into lithium-heparin coated vacuum tubes. Specimens were centrifuged and the plasma and buffy coat were removed. Sodium and potassium concentrations
in heparinized red cells remain stable for about 4 hours. However, once the hemoglobin lysate has been prepared, it is stable for one month at 4°C or up to one year at -20°C.

3. Test Procedure

Erythrocyte sodium and potassium was measured using a modification of the procedure of Fortes-Mayer and Starkey. a. Wash erythrocytes at least three times by suspending in magnesium chloride solution, centrifuging for 2 minutes to pack the cells and aspirating the supernatant.
b. Mix the washed cells gently and remove a capillary sample for microhematocrit determination.
c. Lyse the cell suspension by adding 10 μl of saponin solution and vortexing briefly.
d. Calibrate the flame photometer using the standard electrolyte solution. Use the 0-200 mmol/L scale for potassium measurement.
e. Analyze each lysate and record results.

4. Calculations

Erythrocyte sodium and potassium concentration is calculated using the formula:

\[
\frac{\text{Recorded } \frac{\text{Na}^+}{\text{K}^+}}{\text{Na}^+ / \text{K}^+ \text{ in mmol/liter RBC}} = \frac{\text{Na}^+ / \text{K}^+}{\text{RBC Hct/100}}
\]

C. Test Population

Erythrocyte \(^{22}\text{Na}\) influx and intracellular sodium and potassium was determined on 105 consenting subjects. No restrictions were imposed regarding fasting state, diet, alcohol or
caffeine intake. Individuals were divided into four groups as follows:

1. **Group I (Normotensive Controls)**

   The control group consisted of 25 apparently healthy, caucasian adults (10 men and 15 women) with normal blood pressure (diastolic pressure less than 90 mm Hg) ranging in age from 23-45. All individuals were students or staff members of the Department of Pathology and Laboratory Medicine. There was no history of hypertension in the parents of this group.

2. **Group II (Normotensive with Family History of Hypertension)**

   This group comprised 18 Caucasian adults (7 men and 11 women) from 23 to 48 years of age who had at least one parent with high blood pressure. All subjects were apparently healthy and had normal blood pressure at time of sampling.

3. **Group III (Essential Hypertension)**

   This group consisted of 25 Caucasian adults (15 men and 10 women) with mild to moderate essential hypertension, having a diastolic pressure greater than 95 mm Hg before treatment. Secondary hypertension was excluded by normal laboratory renal function tests. All patients were receiving anti-hypertensive medications including thiazide diuretics, beta-blockers and angiotensin-converting-enzyme inhibitors. A family history of hypertension was reported by 17 patients.

4. **Group IV (Secondary Hypertension)**

   This group of 25 patients comprised 13 men and 12 women from 11 to 59 years of age with hypertension secondary to renal
disease. All were being treated with anti-hypertensive and/or immunosuppressive medications. Five patients reported a family history of high blood pressure.

D. Statistical Evaluation

The unpaired Student t test was used to evaluate the data from all groups. Differences were accepted as significant if a p value of less than 0.05 was found.
IV. RESULTS

A. Sodium-22 Influx Study

1. Procedure Validation

Linearity of the $^{22}\text{Na}$ influx procedure was found to be constant for at least four hours (Figure 2). The correlation coefficient between measured $^{22}\text{Na}$ uptake and time exceeded 0.99.

Day-to-day precision of the method was assessed by analyzing a control specimen for five consecutive days. Cells were studied fresh and after storage at $4^\circ\text{C}$ for 24-hour periods. These results yielded a day-to-day coefficient of variation of 3.1% and demonstrated that sodium influx into red cells is not affected by storing at $4^\circ\text{C}$ for up to five days.

The same control subject was studied over a period of five weeks (seven different specimens) to determine individual variation and test reproducibility. In addition, five patients were randomly repeated at least four weeks after the initial assay. Little variation occurred in either the test itself (CV: 5.7%) or in individual values (Table 2), suggesting that sodium influx remains constant over a period of weeks in cells from the same donor.

2. Clinical Study

Mean values and ranges of sodium influx for the four groups are shown in Table 3 and Figure 3. Individual male and female values are illustrated as a scatter diagram in Figure 4.
FIGURE 2: LINEARITY OF THE $^{22}\text{Na}$ INFLUX PROCEDURE

$R = 0.99$
# TABLE 2: INDIVIDUAL VARIATION IN SODIUM-22 INFLUX

Patients were repeated at least 4 weeks after the initial determination

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>INITIAL RESULT*</th>
<th>REPEAT RESULT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.208</td>
<td>0.232</td>
</tr>
<tr>
<td>2</td>
<td>0.270</td>
<td>0.272</td>
</tr>
<tr>
<td>3</td>
<td>0.130</td>
<td>0.153</td>
</tr>
<tr>
<td>4</td>
<td>0.321</td>
<td>0.300</td>
</tr>
<tr>
<td>5</td>
<td>0.208</td>
<td>0.198</td>
</tr>
</tbody>
</table>

* mmol/liter cells/hour
TABLE 3: SUMMARY OF RESULTS: Sodium-22 Influx and Intracellular Sodium and Potassium in the Four Groups Studied (*p < 0.05; **p<0.01; ***p<0.001)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>N</th>
<th>22Na INFLUX (mmol/L cells/hr)</th>
<th>Na⁺ (mmol/L cells)</th>
<th>K⁺ (mmol/L cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
</tr>
<tr>
<td>I</td>
<td>25</td>
<td>0.216</td>
<td>0.037</td>
<td>7.1</td>
</tr>
<tr>
<td>Normotensive</td>
<td>M</td>
<td>10</td>
<td>0.2229</td>
<td>0.042</td>
</tr>
<tr>
<td>Controls</td>
<td>F</td>
<td>15</td>
<td>0.212</td>
<td>0.036</td>
</tr>
<tr>
<td>II</td>
<td>18</td>
<td>0.240</td>
<td>0.057</td>
<td>7.1</td>
</tr>
<tr>
<td>Normotensive</td>
<td>M</td>
<td>7</td>
<td>0.225</td>
<td>0.060</td>
</tr>
<tr>
<td>with FH of</td>
<td>F</td>
<td>11</td>
<td>0.230</td>
<td>0.056</td>
</tr>
<tr>
<td>hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>25</td>
<td>0.278</td>
<td>0.079</td>
<td>7.7</td>
</tr>
<tr>
<td>Essential</td>
<td>M</td>
<td>15</td>
<td>0.293</td>
<td>0.078</td>
</tr>
<tr>
<td>Hypertension</td>
<td>F</td>
<td>10</td>
<td>0.255</td>
<td>0.079</td>
</tr>
<tr>
<td>IV</td>
<td>25</td>
<td>0.220</td>
<td>0.047</td>
<td>6.1***</td>
</tr>
<tr>
<td>Secondary</td>
<td>M</td>
<td>13</td>
<td>0.222</td>
<td>0.040</td>
</tr>
<tr>
<td>Hypertension</td>
<td>F</td>
<td>12</td>
<td>0.218</td>
<td>0.054</td>
</tr>
</tbody>
</table>
FIGURE 3: MEAN VALUES AND RANGES OF SODIUM-22 INFLUX IN FOUR GROUPS OF SUBJECTS

GROUP I - NORMOTENSIVE CONTROLS
GROUP II - NORMOTENSIVE WITH FAMILY HISTORY OF HYPERTENSION
GROUP III - ESSENTIAL HYPERTENSION
GROUP IV - SECONDARY HYPERTENSION

$P < 0.01$
FIGURE 4: SODIUM-22 INFLUX IN FOUR GROUPS OF SUBJECTS, SUBCLASSIFIED BY SEX (M: ▲, F: ●)

GROUP I - NORMOTENSIVE CONTROLS
GROUP II - NORMOTENSIVE WITH FAMILY HISTORY OF HYPERTENSION
GROUP III - ESSENTIAL HYPERTENSION
GROUP IV - SECONDARY HYPERTENSION

22Na INFLUX (MMOL/LITER CELLS/HOUR)

I  II  III  IV
Mean sodium-22 influx was significantly higher ($p < 0.01$) in erythrocytes from essential hypertensives (Group III) (0.278 ±0.079 mmol/liter cells/hour) than from normotensive controls (Group I) (0.216 ±0.037) or secondary hypertensives (Group IV) (0.220 ±0.047) (Figure 3). This difference between groups was observed for both males and females. A comparison of male and female values within each group revealed no significant sexual bias, although males had higher mean values in all groups studied (Figure 5).

Fourteen of 25 essential hypertensive patients had values within 2 SD of the normal mean value (Figure 4). The observed test sensitivity was 44%. However, all secondary hypertensives had results within the control range, indicating an observed specificity of 100%.

A genetic influence on red cell sodium influx was not observed in this study. Fifteen of 18 normotensive offspring of essential hypertensive parents (Group II) had normal values. Eighteen patients with essential hypertension and five patients with secondary hypertension reported having a family history of high blood pressure. However, no direct correlation could be established between degree of $^{22}$Na influx and a family history of hypertension.

B. Intracellular Sodium and Potassium

Precision of the method was assessed by repeated analysis of a random lysate specimen with each batch of unknown samples. These replicate results yielded a CV of 1.4% for Na$^+$ and 1.5%.
FIGURE 5: COMPARISON OF MEAN MALE AND FEMALE SODIUM-22 INFLUX VALUES

GROUP I - NORMOTENSIVE CONTROLS
GROUP III - ESSENTIAL HYPERTENSION
for $K_i^+$. Specimens stored at $4^\circ C$ were found to be stable for at least one month.

Mean values for intracellular sodium and potassium are shown in Table 3 and in Figures 6 and 8. No difference in $Na_i^+$ or $K_i^+$ was observed between normal controls (Group I) and essential hypertensives (Group III). However, secondary hypertensives (Group IV) showed a significantly lower $Na_i^+$ and higher $K_i^+$ when compared to any group studied ($p<0.001$).

Comparison of male and female values within each group revealed unexpected differences (Figure 7). Male controls had higher $Na_i^+$ than female controls ($p<0.05$), not only in Group I, but also in Group II where there was a family history of hypertension. Potassium values were essentially the same for men and women in these groups. Male essential hypertensives also had higher $Na_i^+$ than female hypertensives ($p<0.01$). In addition, males of this group showed a significantly lower $K_i^+$ than females ($p<0.05$). No differences in $Na_i^+$ or $K_i^+$ were seen between males and females with secondary hypertension.

Intracellular $Na^+$ and $K^+$ do not appear to influence $^{22}Na$ influx. A comparison of individual $Na_i^+$, $K_i^+$ and $^{22}Na$ influx values for each group failed to show a significant correlation among these parameters ($r=-0.17$).
FIGURE 6: COMPARISON OF MEAN INTRACELLULAR SODIUM CONCENTRATIONS FOR THE FOUR GROUPS STUDIED

GROUP I - NORMOTENSIVE CONTROLS
GROUP II - NORMOTENSIVE WITH FAMILY HISTORY OF HYPERTENSION
GROUP III - ESSENTIAL HYPERTENSION
GROUP IV - SECONDARY HYPERTENSION
FIGURE 7: COMPARISON OF INTRACELLULAR SODIUM CONCENTRATIONS SUBCLASSIFIED BY SEX

GROUP I - NORMOTENSIVE CONTROLS
GROUP III - ESSENTIAL HYPERTENSION

\[ \text{Na}^+ \] (mmol/liter cells)

\[ P < 0.01 \]

\[ P < 0.05 \]
FIGURE 8: COMPARISON OF MEAN INTRACELLULAR POTASSIUM CONCENTRATIONS FOR THE FOUR GROUPS STUDIED

GROUP I - NORMOTENSIVE CONTROLS
GROUP II - NORMOTENSIVE WITH FAMILY HISTORY OF HYPERTENSION
GROUP III - ESSENTIAL HYPERTENSION
GROUP IV - SECONDARY HYPERTENSION

$P < 0.001$
V. DISCUSSION

A. Sodium-22 Influx Study

This study was prompted by the work of Mahoney et al. who described a simple and reproducible laboratory test for identifying patients with essential hypertension. Erythrocytes from these patients showed a significantly greater influx of sodium ions when compared to normotensives or secondary hypertensives. The present study is only in partial agreement with their results. The increase in sodium uptake in red cells from essential hypertensives was significant but of a different magnitude than that reported by Mahoney et al. The mean influx value for essential hypertensives was almost 50% lower in this study. Normals and secondary hypertensives also showed generally lower results but the differences were not as great.

Two telephone conversations with Dr. Mahoney failed to reveal any obvious technical disparities. Differences in counting efficiency between scintillation counters was considered, but this factor does not adequately explain the disparity.

These findings emphasize the necessity for each laboratory to establish its own range of values for a particular patient population.

Overlap occurred in all groups studied. If arbitrary limits were established, using the mean ±2 SD of the normal population, then only 11 of 25 (44%) essential hypertensives studied would have been considered to have a positive result.

Mahoney et al. showed almost no overlap between essential hyper-
tensives and normals or secondary hypertensives. It is difficult to determine the reason for this difference. Considerable variation in red cell transport capacity of apparently healthy persons has been previously demonstrated.\textsuperscript{26} The observed overlapping of results may be due to this variation, as well as possible demographic differences in patient populations.\textsuperscript{9} It must also be realized that essential hypertension may have multiple etiologies with varying degrees of sodium transport function.

An interesting aspect of sodium transport tests is the possibility of identifying persons who are at risk for developing hypertension. Mahoney et al.\textsuperscript{29} indicates that about 50\% of offspring of hypertensive parents will demonstrate increased red cell Na\textsuperscript{+} permeability. Garay\textsuperscript{17} and Canessa\textsuperscript{8,9} also report positive erythrocyte tests in children of parents with essential hypertension. Their observations could not be clearly demonstrated in this present study. Only three of 18 first-degree offspring of patients with essential hypertension could be identified as having a positive test by this method. Again, overlapping of test results and individual variation might mask some persons with the erythrocyte defect. Possibly test parameters need to be altered to improve sensitivity. Henningsen and Nelson\textsuperscript{25} found that \textsuperscript{22}Na uptake by red cells was the same in controls and in offspring of essential hypertensive patients after 80 minutes incubation, but was significantly higher in red cells of offspring after 140 minutes.
Sex differences in red cell sodium transport have been reported by several authors. A statistically significant elevation of sodium-lithium countertransport in male hypertensive patients was found by both Canessa and Ibsen. Female hypertensives also showed elevated results but much less than males. The results of the current investigation support this concept. Although the differences found were not significant, males had higher mean *22Na influx values in all groups studied. Males were also more likely to have higher individual values. Of 11 essential hypertensive patients with elevated influx values, eight were males. It is not known whether these observed differences are of genetic, environmental, or endocrine origin. Recent evidence suggests that a genetic sex linkage may exist, or endocrine factors may modulate the genetically determined sodium transport systems, which may explain why Ibsen et al. failed to find an elevation in sodium-lithium countertransport in pre-pubertal children. Worley et al. found that countertransport was elevated during pregnancy, both for normotensive and hypertensive women, strongly suggesting an endocrine influence, perhaps on erythropoiesis, since sodium transport functions are likely to be determined at this time. The study of Worley et al. suggests that physiologic changes may temporarily alter sodium transport activity, important to consider if erythrocyte tests are to be applied to the diagnosis of essential hypertension. Williams found a sig-
significant correlation between serum cholesterol concentration and sodium-lithium countertransport in non-pregnant subjects. Since cholesterol is an important cell membrane component, its availability during membrane formation could influence the function of sodium transport proteins. During the present study, two patients were excluded due to hypercholesterolemia. Both of these patients were normotensive and had elevated $^{22}$Na influx values. One additional subject was excluded because she was 26 weeks pregnant. Her $^{22}$Na influx was also elevated. These and other potential physiologic influences need further investigation.

Racial factors also influence the activity of sodium transport systems. Etkin et al. found that Blacks completely lack the abnormal Na$^+$ transport characteristic of Caucasian essential hypertensives. Sodium-lithium countertransport is also lower in Black hypertensives than in Caucasians. This author investigated $^{22}$Na influx in 12 Black essential hypertensive patients (2 men and 10 women) and found that Black hypertensives had significantly lower ($p < 0.001$) erythrocyte Na$^+$ influx than white hypertensives. These data (not shown) support the hypothesis that essential hypertension may have a different expression in Blacks.

The Na$^+$ influx method reported here correlates well with other published techniques. Uptake of $^{22}$Na by red cells involves mostly passive permeability but may also reflect other ouabain-insensitive pathways. Mahoney et al. found a strong association between this method and sodium-lithium
countertransport. From a clinical standpoint, however, the technique reported here is superior. It is simple, rapid and requires little expertise. Sensitivity of the method is variable but specificity is high. The predictive value of a positive test, as observed in this study, approaches 100%. The procedures developed by Garay et al. and Canessa et al. are lengthy, cumbersome and not well suited to routine laboratory use. This author has attempted the cotransport assay of Garay and found it complicated and prone to error.

An important laboratory parameter to control in this procedure is the amount of $^{22}\text{Na}$ in the incubation mixture. The nature of the calculations is such that small errors in scintillation counting may produce large errors in the calculated sodium influx. Best results were obtained with a $^{22}\text{Na}$ activity of about 200,000 cpm/0.5 ml incubation media. Isotope should be purchased in sufficient quantity so that a large, standardized batch of media can be prepared, aliquoted and frozen for future use.

Osmolality and pH of buffer solutions and incubation temperature should also be controlled. Swelling of red cells inhibits $\text{Na}^+\text{,K}^+$-cotransport, and $\text{Na}^+,\text{Li}^+$-countertransport is sensitive to pH changes. Since the exact molecular basis of $\text{Na}^+$ influx in this procedure is unknown, it seems prudent to maintain physiologic conditions at all times.
B. Intracellular Sodium and Potassium

The results of this study show that the sodium and potassium concentrations of erythrocytes of patients with essential hypertension are not different from those of normal controls. The data agree with the findings of Walter and Distler\textsuperscript{43} and Canessa et al.\textsuperscript{8} but they contrast with the work of Aderounmu and Salako\textsuperscript{1}, who found increased erythrocyte sodium in essential hypertensive African Blacks. The discrepancy may be due to racial differences. This author found red cell sodium content to be significantly higher ($p < 0.05$) in 12 Black patients with essential hypertension than in 25 white patients ($9.6 \pm 3.2$ vs $7.7 \pm 1.7$ mmol/liter cells). Although sufficient Black controls were not available for comparison, these results seem to support the concept of racial factors influencing cell sodium metabolism.

Patients with secondary hypertension showed an unexpected lower erythrocyte sodium and higher potassium when compared to either normal controls or essential hypertensives. The reason for these differences in intracellular cations is unclear. Both groups of hypertensive patients were taking antihypertensive medications, including thiazide diuretics. Only the secondary hypertensives were on steroids (prednisone or prednisolone) as part of post-transplant therapy, but this factor seems unlikely to cause intracellular sodium depletion and potassium retention due to the expected mineralocorticoid effect on extracellular sodium and potassium. Furosemide and
propranolol would also affect these ions in a similar manner. A follow-up study may be needed to determine if these observations are a non-specific artifact or directly related to some exogenous influence.

Unexpected sex-related differences in intracellular sodium and potassium were also noted. Both male controls and male hypertensives had higher Na\(^+\) than their female counterparts. These results conflict with those of Fortes Mayer and Starkey who found erythrocyte Na\(^+\) and K\(^+\) to be the same in both sexes. Male patients with essential hypertension also exhibited a lower red cell K\(^+\) than females. At present these findings cannot be explained.

Henningsen and Nelson have suggested that net influx of sodium is dependent on the intracellular concentration of sodium. They found a significant positive correlation between these two parameters in normotensive offspring of essential hypertensive parents. The results of the present study do not support this conclusion. Individual values for Na\(^+\) influx and intracellular Na\(^+\) were compared for all groups studied. No correlation was found in any group, the slope of the regression line being nearly zero.

The technique reported here for measuring erythrocyte sodium and potassium represents a modification of the method of Fortes Mayer and Starkey. Results obtained using this method compare well with previously published work. The technique is simple, reproducible and suitable for use in the clin-
ical laboratory. Manual pipetting and diluting steps are eliminated, thereby greatly simplifying the procedure and reducing sources of error. The viscous nature of the lysate requires the use of a positive displacement automatic pipet; peristaltic pumps (e.g. Model IL144, Instrumentation Laboratories Inc., Lexington, MA 02173) are unsuited for this step. The use of saponin for cell lysis is quicker and more efficient than methods involving osmotic shock or freezing. Although saponin contains appreciable amounts of sodium and potassium, the amount of either cation in a 10 µl aliquot is negligible.
VI. SUMMARY

A simple new procedure to measure erythrocyte uptake of $^{22}\text{Na}$ was evaluated as a laboratory test for the diagnosis of essential hypertension. Erythrocytes from 25 normotensive controls, 18 normotensive offspring of essential hypertensive parents, 25 patients with diagnosed essential hypertension, and 25 patients with hypertension secondary to renal disease, were incubated in an isotonic buffer containing $^{22}\text{Na}$. Intracellular sodium and potassium were also measured to determine the influence of these cations on net sodium influx.

Uptake of $^{22}\text{Na}$ by erythrocytes is linear for at least four hours. A day-to-day variation of 3.1% can be achieved using this procedure. Sodium influx remains constant over a period of weeks in the same individual. Method sensitivity, as observed in this study, was 44%. However, specificity and the predictive value of a positive test are almost 100%.

Sodium-22 influx was significantly higher in patients with essential hypertension than in normal controls or secondary hypertensives. Male normotensive controls and patients in all groups had higher mean influx values than females, but the differences were not significant.

Overlap occurred in all groups studied. Fifteen of 18 (83%) normotensives with a family history of hypertension showed normal results. Fourteen of 25 (56%) essential hypertensives also had normal results. A positive family history for hypertension was not correlated with degree of sodium influx.
Erythrocyte sodium and potassium concentration in patients with essential hypertension is not significantly different from normal controls. However, secondary hypertensives in this study showed a lower Na\textsuperscript{+} and higher K\textsubscript{i} than any other group. This observation may be drug related. Male controls and male essential hypertensives had higher intracellular sodium than females. Males with essential hypertension also showed a lower K\textsubscript{i} than females.

Sodium influx does not appear to be affected by the intracellular concentration of Na\textsuperscript{+} or K\textsuperscript{+}. The correlation coefficient between these parameters was -0.17.
ACKNOWLEDGEMENTS

I am grateful to the Department of Pathology and Laboratory Medicine at University Hospital for supporting me in all phases of this project. I especially thank the staff of Special Chemistry for making me feel welcome, lending technical advice and even cheerfully donating blood specimens for my study.

Special thanks go to my advisors, Mary C. Haven and Dr. Arden E. Larsen, for their encouragement, support and guidance in preparing this thesis.

I would also like to thank Dr. Robert Grissom and the staff of the Hypertension Clinics for obtaining patients' specimens and data for the project.

In addition, thanks is given to Dr. Richard Steenburg and the staff of the Renal Transplant Department at Bishop Clarkson Memorial Hospital for providing blood specimens and information about the patients.

I am likewise grateful to Dr. Donald Darst for providing hypertensive patients for this study.

My graduate studies program was supported by the United States Air Force Institute of Technology. I am very thankful to those who selected me for this rewarding opportunity.

Finally, thanks go to my wife, Mary, and my sons, Jason and Jeffrey, for being patient and understanding during my whole graduate program.
REFERENCES


ERYTHROCYTE SODIUM TRANSPORT IN ESSENTIAL HYPERTENSION

PATIENT CONSENT FORM

You have been asked to participate in a study that may help in diagnosing high blood pressure. In this study a measurement of your sodium is made of the blood sample in the laboratory. Your decision to participate, at no charge to you, will require a confidential history and a blood sample obtained by routine venipuncture technique. No injection of any medication is made. There are only minimal risks including a small amount of pain and possible bruising. The amount of blood drawn is about two teaspoons or 10cc. Any information obtained in connection with the study will be strictly confidential.

Your decision to whether or not to participate will not prejudice your future relations with the University of Nebraska Hospital. If you decide to participate at any time without prejudice. Any questions concerning this study should be directed to Dr. Grissom at 559-5151 or Mr. Doug Sargent at 559-4255. You will be given a copy of this form to keep.

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE. YOUR SIGNATURE INDICATES THAT YOU HAVE DECIDED TO PARTICIPATE AND HAVE READ AND UNDERSTOOD THE INFORMATION PROVIDED ABOVE.

Date ___________________________ Patient's Signature ___________________________

Witness ___________________________ Investigator ___________________________
ERYTHROCYTE SODIUM TRANSPORT IN ESSENTIAL HYPERTENSION

PROCEDURE: One consent form to chart and give one copy to patient. Call Doug Sargent at 4255 and complete this form. Send patient to outpatient laboratory to have 10cc heparinized blood sample drawn.

NAME: BLOOD PRESSURE:
AGE/SEX: HEIGHT:
RACE: WEIGHT:
CURRENT MEDICATIONS: (Please include all medications)

ANY HISTORY OF HIGH BLOOD PRESSURE IN PATIENT OR FAMILY?
Patient ____ Parents ____ Paternal Grandparents ____
Siblings ____ Children ____ Maternal Grandparents ____

HAS PATIENT INGESTED ALCOHOL WITHIN LAST 24 HOURS?
COFFEE, TEA OR OTHER CAFFEINATED BEVERAGE?

DIAGNOSIS: Essential ____ Secondary ____ Normotensive ____

COMPLICATIONS OR OTHER PROBLEMS?

If there are any questions, please contact Dr. Grissom at 5151 or Doug Sargent at 4255.