Cation Imbalance in Erythrocytes, Serum and 24-hour Urine from Patients with Essential Hypertension and Adolescents with High Blood Pressure

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Electrolyte concentrations in the erythrocytes, serum and 24-hr urine were measured in 34 untreated essential hypertensives (EH), 32 normotensives with a family history of hypertension (N(F)), 112 nomotensives without a family history of hypertension (N(F)), 76 senior high school students with high blood pressure (H) and 110 students with normal blood pressure (C). For the measurement of intra-erythrocyte electrolytes, a new method to decrease the trapped plasma was developed and adopted. A new simple method to collect 24-hr urine was devised, too. The results were as follows: 1) The sodium concentration [Na] and the ratio of [Na] to potassium concentration [Na/K] in the erythrocytes were significantly higher in the EH and N(F) than in the group of N(F) (p < 0.01). 2) There was a positive correlation (r = 0.43) between the [Na/K] in the serum and erythrocytes in the 3 groups. The [Na/K] value in the erythrocytes to a given [Na/K] value in the serum was higher in EH than in the group of N(F). 3) When the group of H was compared with C, the Na excretion of the 24-hr urine in the former group was found to be slightly higher, and the [Na/K] of the 24-hr urine and the [Na] and [Na/K] values of the erythrocytes were significantly higher, too.

It may therefore be said that an increase in the intracellular [Na] or [Na/K], which may be caused genetically, and an excess of Na intake and an increase of the ratio of Na/K intake are presumed to play an important role in the pathogenesis of essential hypertension.

It has been generally accepted that essential hypertension is caused by a combination of genetic and environmental factors, and among the latter, an excess of sodium intake and the increased ratio of sodium intake to potassium have been considered to be most important. Several studies done to correlate cell sodium with arterial hypertension have indicated that erythrocytes from patients with essential hypertension or from some of their offspring are abnormal. The changes in the red blood cell (RBC) included a rise in the sodium concentration and an inherited defect in a Na\(^+\)-K\(^+\) co-transport, an increase in the one-to-one $L^\prime$ : Na\(^+\) counter-transport, and a high unidirectional influx and efflux of Na\(^+\).
TABLE I

<table>
<thead>
<tr>
<th></th>
<th>Normotensives</th>
<th>Essential hypertensives</th>
<th>Secondary Hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-)</td>
<td>(+)</td>
<td>Untreated</td>
</tr>
<tr>
<td>No. of Cases (male/female)</td>
<td>112 (62/50)</td>
<td>32 (25/7)</td>
<td>34 (19/15)</td>
</tr>
<tr>
<td>Age (yrs old)</td>
<td>34 ± 13</td>
<td>34 ± 10</td>
<td>46 ± 10</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>117 ± 10</td>
<td>128 ± 14</td>
<td>176 ± 22</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>71 ± 11</td>
<td>78 ± 11</td>
<td>106 ± 14</td>
</tr>
</tbody>
</table>

(-) = without a family history of hypertension, (+) = with a family history of hypertension

mean ± SD

However, in other studies, the concentration of sodium in RBC did not increase significantly in essential hypertensives. The normal values of intra-erythrocyte electrolytes have varied considerably among the studies, and such variances were considered to be attributable to either an error in the determination or the treatment of trapped plasma.

We have developed a new method to decrease the trapped plasma in the process of measuring intra-erythrocyte electrolytes, and also a simple device useful for the collection of 24-hr urine. Using these methods, the concentration of sodium [Na] and that of potassium [K] of the erythrocytes, serum and 24-hr urine were reanalyzed in hypertensives and normotensives with or without a family history of hypertension. The aim of such examinations was to see if there exists a certain general abnormality of the balance of intracellular electrolytes, extracellular electrolytes and the ingested amount of electrolytes in essential hypertensive patients and their offspring. It was also discussed whether such an abnormality would be seen in adolescents with hypertension, and if so, whether it would affect the progress of essential hypertension.

MATERIALS AND METHODS
Estimation of Intra-erythrocyte Electrolytes
Ten to 15 ml of blood samples were put into heparinized tubes and centrifuged immediately...
at 3000 rpm (or 2,240 × g) for 30 min to separate plasma and the buffy coat from the sediment. The sediment containing erythrocytes was divided into two parts. One part was completely hemolyzed using an ultrasonic apparatus which we devised and the hemolysate was transferred to a plastic tube. Another part, of the same quantity of sediment, which contained erythrocytes, was put into the plastic tube containing hemolysate. The mixture was centrifuged at 10,000 rpm (or 10,550 × g) for 20 min. Then, the central part of the tube in which sediment was contained, was cut away to take out the packed erythrocytes. The [Na] and [K] were measured with a flame photometer (Hitachi Model 775).

Figure 1 shows the apparatus used for hemolyzing with supersonic waves. It has a stainless steel resonator in its stainless steel test tube. The tube is sealed hermetically after putting blood into it, and supersonic shock waves directed at it from the outside. It is capable of inducing 2 ml of blood hemolysis within 3 min and many samples can be prepared at a time.

The mean coefficients of variation (CV) for 14 samples were 1.99 ± 1.41 (SD) % for [Na] and 0.47 ± 0.44% for [K]. Likewise, the CVs for 5 examinees, when measurements were done more than twice within 3 months, were 4.33 ± 1.98% for [Na] and 1.90 ± 0.82% for [K].

Estimation of Electrolytes in Urine Collected during 24-hr by Proportionally Sampling Method

We have devised a new urine sampling method, which we called “proportional urine sampling”. This facilitates the collection of 24-hr urine at the outpatient clinic or in mass screenings. The total content of electrolytes discharged into the urine during a 24-hr period can easily be estimated by this method collecting a fixed rate of urine.

This is a double-bottomed partitioned-cup, which is 6.5 cm in diameter and 14 cm in height. It has a pipe-shaped scale, which runs vertically along the wall of the cup, and has a cock in its lower end leading to the lower compartment of the cup. The cup is so devised that 1% of the total urine volume excreted into the upper compartment each time remains in the pipe-shaped scale, and then comes down into the lower compartment when the cock is pulled out. After this, the urine remaining in the upper compartment can be discarded. By repeating this procedure, urine can be collected over a 24-hr period. That is, the total amount of urine excreted in a day can be determined by multiplying the total volume of urine accumulated in the lower compartment by 100. Since the concentration of electrolytes would be the same regardless of whether it is determined in 1% of the urine or in the total, a portion of the urine is therefore sufficient. Thus the measurements of Na, K and creatinine content were done in the urine gathered in the lower compartment of the cup, and the values so obtained were multiplied by 100.

All examinees may carry the cups in vinyl bags if they wish, so that it is possible to collect urine even during working hours. These cups can be used also in the mass screenings and outpatient clinic examinations at different places. The correlation coefficient of the relationship between 24-hr urine volume and the amount of urine collected using the partitioned-cup was r = 0.98, and the average variation was 0.05 ± 0.15 L/day, according to the results of tests on 32 examinees. Similarly, the correlation of Na excretions was r = 0.99, and the average variation was 0.40 ± 5.3 mmol/day. The correlation of K excretions was r = 0.99, and the average variation was 0.1 ± 2.5 mmol/day.

Subjects

Five adults and 3 adolescents groups were set up. For the adult (Table I), the normotensive group consisted of 112 normotensives which were selected from hospital workers who did not have hypertensive parents, siblings or grand parents. The normotensive group consisted of 32 normotensives in whose families at least one parent had suffered from hypertension. Patients with essential hypertension were divided into two groups, untreated and treated. The former group consisted of 20 outpatients and 14 inpatients, both of which had been proved to be free from secondary hypertension based on clinical, biological and radiological examinations before the pharmacotherapy. The latter group consisted of 20 outpatients who were treated with such antihypertensives as thiazide, β-blocker and α-methyldopa. The group of 12 secondary hypertensive included 9 with renal failure resulting from chronic glomerulonephritis, 2 with renovascular hypertension and one case with Cushing’s syndrome. Of these patients, 3 had at least one hypertensive parent, but they had no other hypertensive relatives. The age, sex and number of group members as well as their blood pressures are summarized in Table I.

Fig. 2. Two hundred and sixteen adolescents with high blood pressure (systolic BP > 135 mmHg) and 111 normotensive control group members (systolic BP < 130 mmHg) were selected from among 2,973 senior high school students, and their 24-hr urines were collected and examined. The H group consisted of 76 students whose systolic blood pressures were higher than 140 mmHg at 3 times of measurement. The B and C groups consisted of 97 students (140 mmHg > systolic BP > 130 mmHg) and 110 students (systolic BP < 130 mmHg), respectively.

2,973 public senior high school students. They collected 24-hr urine samples by the proportional urine sampling method and were divided into two groups, H and B. The H group consisted of 76 (69 male and 7 female) hypertensive students who had exhibited blood pressure as high as 140 mmHg or more in 3 measurements. The B group consisted of 97 (73 male and 24 female) with systolic blood pressure exceeding 130 mmHg but not reaching 140 mmHg in 3 measurements. The students whose 24-hr urinary data were unreliable were excluded. The C group consisted of 110 (76 male and 34 female) who had been selected at random from the students whose blood pressure was less than 130 mmHg.

Data were analyzed by means of the product-moment correlation coefficient, Student’s t-test and Kai-square test.

RESULTS

[Na] in the Erythrocytes of Normotensives
The mean Na in erythrocytes was 8.8 ± 0.9 (SD) mmol/L-RBC in the normotensive group, but was 10.5 ± 1.1 mmol/L-RBC in the normotensive group (p < 0.01) (Fig. 3). If a borderline could be set at 10 mmol/L-RBC, approximately 90% of the subjects in the normotensive group were found below this level, and more than 63% of those in the normotensive group were above it (p < 0.01).

[Na] in the Erythrocytes of Hypertensives
The [Na]’s in the erythrocytes, as expressed
TABLE II  SODIUM AND POTASSIUM CONCENTRATIONS IN THE RBC OF NORMOTENSIVES AND HYPERTENSIVES

<table>
<thead>
<tr>
<th></th>
<th>Normotensives</th>
<th>Essential hypertensives</th>
<th>Secondary hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(+)</td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Cases</td>
<td>112</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>M.C.V. of RBC (μL)</td>
<td>90.3 ± 4.7</td>
<td>92.2 ± 6.5</td>
<td>90.7 ± 4.3</td>
</tr>
<tr>
<td>Concentrations in RBC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Na] (mmol/L-RBC)</td>
<td>8.8 ± 0.9</td>
<td>10.5 ± 1.1**</td>
<td>10.3 ± 1.0**</td>
</tr>
<tr>
<td>[K] (mmol/L-RBC)</td>
<td>96.0 ± 3.4</td>
<td>94.7 ± 2.9**</td>
<td>93.2 ± 4.1**</td>
</tr>
<tr>
<td>[Na/K]</td>
<td>0.091 ± 0.012</td>
<td>0.111 ± 0.017**</td>
<td>0.111 ± 0.014**</td>
</tr>
</tbody>
</table>

M.C.V. = mean corpuscular volume, * = p < 0.05, ** = p < 0.01 vs normotensives (-)

TABLE III  CORRELATION COEFFICIENTS BETWEEN THE SERUM AND INTRAERYTHROCYTE ELECTROLYTES

<table>
<thead>
<tr>
<th></th>
<th>Normotensives</th>
<th>Essential hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(+)</td>
<td>Untreated</td>
</tr>
<tr>
<td>No. of cases</td>
<td>76</td>
<td>23</td>
</tr>
<tr>
<td>[Na] in serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-[Na] in RBC</td>
<td>-0.06</td>
<td>0.27</td>
</tr>
<tr>
<td>-[K] in RBC</td>
<td>-0.07</td>
<td>0.30</td>
</tr>
<tr>
<td>-[Na/K] in RBC</td>
<td>-0.11</td>
<td>-0.27</td>
</tr>
<tr>
<td>[K] in serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-[Na] in RBC</td>
<td>-0.32*</td>
<td>-0.20</td>
</tr>
<tr>
<td>-[K] in RBC</td>
<td>0.14</td>
<td>0.34</td>
</tr>
<tr>
<td>-[Na/K] in RBC</td>
<td>-0.37**</td>
<td>-0.43*</td>
</tr>
<tr>
<td>[Na/K] in serum</td>
<td>0.43**</td>
<td>0.44*</td>
</tr>
</tbody>
</table>

* = p < 0.05, ** = p < 0.01

by mean values, were significantly higher in both the treated and untreated groups of essential hypertension than in the normotensive group (Fig. 4). Concentrations exceeding 10 mmol/L-RBC were noticed in 65% and 74% of the treated and untreated hypertensives, respectively (p < 0.01).

In the present investigation, no significant difference was observed between the concentrations in the secondary hypertension group and normotensive group.

Mean [Na] obtained in all the groups are summarized in Table II. Despite no differences in the mean corpuscular volumes of erythrocytes among the groups, there were observed differences in Na contents as well as in [Na]s of erythrocytes among the groups.

[K] in the Erythrocytes

The [K] in the erythrocyte in the untreated group of essential hypertension was slightly, but significantly, lower than in the normotensive group (Table III). However, taking the CV into consideration, it may not be sufficient to enable any definite conclusion to be drawn from this result.

The concentration ratios of [Na] to [K] in the erythrocytes were significantly higher in both the group of essential hypertension and in the normotensive group than in the normotensive group (p < 0.01).

Relationship between Electrolyte Concentrations in the Erythrocytes and the Serum

There was no correlation at all between [Na] values in serum and [Na] or [K] values in the erythrocytes in either the normotensive groups or the untreated group with essential hypertension. However, a slight negative correlation coefficient of r = -0.31 (p < 0.01) was found between the [K] value in the serum and the [Na/K] value in the erythrocytes, and a slight positive correlation

*Japanese Circulation Journal  Vol. 46, May 1982*
coefficient of $r = 0.32$ (p < 0.01) existed between the [Na/K] values in the serum and the [Na/K] values in the erythrocytes. When the correlation coefficient was determined in individual groups, it exceeded a level of $r = 0.43$ (p < 0.01) (Table III).

No correlation was found between [Na], [K], or [Na/K] values in the 24-hr urine and [Na], [K], or [Na/K] in the erythrocytes.

**[Na/K] in the Serum and Erythrocytes in Essential Hypertensive Group**

When observation was made on a graph where the [Na/K] values of the erythrocytes were plotted on the abscissa and those of the serum on the ordinate, it was apparent that most subjects (94%) in the untreated group with essential hypertension were distributed in the lower right part of the graph and most (82%) from the normotensive group in the upper left part (p < 0.01) (Fig. 5). It was thus indicated that in the untreated patients with essential hypertension the erythrocyte [Na/K] value to a given [Na/K] value in the serum is higher than in the normotensive group.

**Na and K Excretions into 24-hr Urine in Hypertensive Senior High School Students**

In the male students, the mean Na content excreted into the 24-hr urine was 228 ± 97 mmol/day in the H group, much greater than the 193 ± 76 mmol/day in the C group (p < 0.02) (Fig. 6). The values exceeded 300 mmol/day in 28% and 5% of the male students in the H and the C groups, respectively, with a significant difference between the groups (p < 0.01). The average body weight of the H group was 5–6 kg, heavier than that of the C group (Table IV). When the amount of Na excreted into the 24-hr urine per kg of body weight was calculated, a slightly larger value was obtained in the male students in the H group than in the C group (p < 0.05) (Table IV).

The amounts of K excreted into the 24-hr urine in the male students did not differ significantly between the H and the C groups. But the number of students who excreted more than 1 mmol K / kg was smaller in the H group than in the C (p < 0.01) (Fig. 7).

The [Na/K] value for the 24-hr urine was higher than 5 in 59% and 36% of the male student of the H and the C groups, respectively, and this difference was significant (p < 0.01). The mean [Na/K] values were 5.4 ± 2.1 and 4.5 ± 1.7 in
TABLE IV  SUMMARY OF DATA ON 24-HR URINARY ELECTROLYTES IN SENIOR HIGH SCHOOL STUDENTS

<table>
<thead>
<tr>
<th>Groups</th>
<th>H (£ 140 mmHg)</th>
<th>B (140—130 mmHg)</th>
<th>C (£ 130 mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>No. of Cases</td>
<td>69</td>
<td>7</td>
<td>73</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.9±10.6</td>
<td>57.1±14.1</td>
<td>62.7±9.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.2±5.6</td>
<td>159.2±4.8</td>
<td>170.3±5.2</td>
</tr>
<tr>
<td>Volume of Urine (ml/day)</td>
<td>1028±359</td>
<td>712±201</td>
<td>950±385</td>
</tr>
<tr>
<td>Creatinine (g/day)</td>
<td>1.71±0.47</td>
<td>1.07±0.11</td>
<td>1.55±0.46</td>
</tr>
<tr>
<td>Na (mmol/day)</td>
<td>228±97*</td>
<td>154±57</td>
<td>200±83</td>
</tr>
<tr>
<td>K (mmol/day)</td>
<td>43±15</td>
<td>36±10</td>
<td>41±13</td>
</tr>
<tr>
<td>Na/Weight (mmol/kg)</td>
<td>3.6±1.6</td>
<td>2.7±1.0</td>
<td>3.2±1.4</td>
</tr>
<tr>
<td>K/Weight (mmol/kg)</td>
<td>0.67±0.25</td>
<td>0.53±0.24</td>
<td>0.67±0.26</td>
</tr>
<tr>
<td>Na/K</td>
<td>5.4±2.1</td>
<td>5.4±2.7</td>
<td>5.0±1.9</td>
</tr>
</tbody>
</table>

mean ± SD  * = p < 0.05,  ** = p < 0.01, vs C group

the male students of the H and the C groups, respectively, with a significant difference (p < 0.01) (Table IV, Fig. 8).

[Na/K] in the Erythrocytes, Serum and 24-hr Urine in the Hypertensive Senior High School Students

Electrolytes in the erythrocytes were examined in 22 (18 male and 4 female) students in the H group who had visited the hospital for examination and had always shown systolic blood pressure exceeding 140 mmHg. Another 25 (21 male and 4 female) normotensive students without a family history of hypertension in the C group were also examined.

As shown in the graph in Fig. 9, 64% of the hypertensive students fall in the lower right part of the graph and 84% of the normotensive students in the upper left part (p < 0.01). In addition, it was found that the [Na]s of erythrocytes were higher in the hypertensive group (10.0 ± 0.8 mmol/L/RBC) than in the normotensive group (8.1 ± 0.9) (p < 0.05).

**DISCUSSION**

Normal values for the intra-erythrocyte electrolytes described in many reports have shown a wide range of distribution. The values quoted are between 6.2 and 24 mmol/L for the Na, and between 81 and 104 mmol/L for the K. The intra-erythrocyte Na has been determined in an unwashed sediment, subtracting a constant figure for the trapped plasma. For instance, it has been determined using various kinds of tracers, such as Evans blue and indocyanine green and radioactively marked substances such as I-albumin, C-sucrose and Na. It has been supposed, however, that the values for the trapped plasma which were determined using tracers with a high molecular weight are lower than those determined using tracers with a low molecular weight.

To eliminate error in measuring Na concentrations in the erythrocytes with the trapped plasma, investigators have washed the erythrocytes with isotonic solutions not containing Na, such as choline chloride solutions$^{33}$ and magnesium chloride solutions$^{26,28,34}$ The intra-erythrocyte [Na] levels in normal subjects were estimated to range from 6 to 8 mmol/L$^{24-26,28}$ in such manners. Washing is an excellent way, but it is feared that some sodium are lost in the washing solution$^{21}$ In addition, it is difficult to prepare the washing solution with the same osmolarity as that of the examinee's plasma at all times.

In the present study, erythrocytes were washed with hemolyzed blood from the same examinee, and the mean [Na] value for the erythrocytes, 8.8 mmol/L-RBC in the normotensive O group, is higher than that obtained by the method where the erythrocytes were washed with a magnesium chloride solution. Probably, this is due to the fact that erythrocytes were mixed with hemolyzed blood and that they were centrifuged at 10,000 rpm (or 10,550 x g) for 20 min, about 8% (8.6 ± 0.4) of the hemolyzed blood (about 0.2~0.3% of the trapped plasma) remaining in the sediment as a result.

Hemolyzed blood contains approximately 100 mmol/L of K, higher than the physiological level. If it were used for washing the erythrocytes, it would have an effect on the Na-K transport through the erythrocyte membrane. In a preliminary study, the Na and K concentration of erythrocytes were measured 10, 20, 30 and 60 min after these erythrocytes had been mixed with hemolyzed blood, there was no significant variation that exceeded the CV as described in the Materials and Methods.

It is known from a number of tracer kinetic studies$^{37,38}$ that a low extracellular K inhibits active Na and K transport. Erythrocytes from patients with hypokalemia were found to have gained Na and lost K.$^{39}$ Glynn$^{40}$ had also reported that in the human erythrocyte the relation between the active potassium influx and extracellular potassium concentration. The intra-erythrocyte K content probably reflects to some degree the level of general intracellular K stored, but the level of plasma K does not necessarily reflect the concentration of intra-erythrocyte K$^{41-43}$ The results of the present experiment revealed that there was a slight positive correlation between the [K] of the serum and the erythrocytes, and further that there was a slight
negative correlation between the [K] of the serum and the [Na] of the erythrocytes. Therefore, the [Na/K] of the serum and that of the erythrocytes would be positively correlated. It is therefore presumed that the [K] of the serum may exert an influence on the [Na/K] of the erythrocytes through the intermediation of the Na-K pump. In evaluating the [Na/K] value for the erythrocytes, the [Na/K] value for the serum as an extracellular fluid should be considered.

There have been many reports describing how patients with essential hypertension have a high [Na] level in the erythrocytes. In contrast, it has been stressed in some other papers that there was no change in that concentration. However, it has already been reported that even normotensives have a high [Na] level in the erythrocytes, when members of their families were hypertensive. Henning 

In this, senior high school students occupying the lower part show high blood pressure and an increase in intracellular [Na] and [Na/K]. It is probable that they will suffer from essential hypertension after they become adults. We will make a follow-up study in the future by increasing the number of cases.

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