Erythrocyte Cation Cotransport and Countertransport in Essential Hypertension


SUMMARY We studied erythrocyte cation cotransport and countertransport systems in 21 and 27 patients with essential hypertension, respectively, all of whom were under 50 years of age, had a diastolic blood pressure level greater than 100 mm Hg, and had a family history of hypertension. The following parameters were normal in nearly all patients: total erythrocyte $\text{Na}^+$ and $\text{K}^+$ concentrations, the maximal rate ($V_{\text{max}}$) of inward cotransport, the affinity of cotransport with $\text{Rb}^+$ as the substrate, the net outward cotransport of $\text{Na}^+$ ions, the passive "leak" influx of $\text{Rb}^+$, and the maximal rate of $\text{Li}^+ - \text{Na}^+$ countertransport. Only four patients gave clearly abnormal results; in two the maximal rate of both cotransport and countertransport was double the normal values, while another two patients demonstrated a greater than twofold increase in passive "leak" influx to $\text{Rb}^+$ ions. Most of the patients with moderate to severe essential hypertension in this Australian study were characterized by normal erythrocyte cation fluxes, but a few showed elevation of both cotransport and countertransport of cations. (Hypertension 6: 360-368, 1984)

KEY WORDS • essential hypertension • erythrocyte cation transport • cotransport • countertransport

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FOUR components of $\text{Na}^+$ transport have been characterized in human erythrocytes, three of which are facilitated by membrane carriers. The first of these three is an active cation pump identified with membrane ($\text{Na}^+ + \text{K}^+$)-stimulated adenosine triphosphatase (ATPase) that extrudes $\text{Na}^+$ from the cell and is inhibited by ouabain.$^1$ The second is a passive cotransport of ($\text{Na}^+ + \text{K}^+$) ions that mediates an equimolar flux of both ions across the membrane by a bidirectional mechanism. This transport system is inhibited by the loop diuretics furosemide and bumetanide.$^2$ The third is $\text{Na}^+ - \text{Na}^+$ exchange diffusion, which occurs by a mechanism that also mediates the countertransport of $\text{Li}^+$ for $\text{Na}^+$ ions.$^3$ Finally, $\text{Na}^+$ ions may cross the membrane by a simple passive leak, the magnitude of which is directly proportional to the concentration gradient for this cation.

An increase in the maximal rate of $\text{Li}^+ - \text{Na}^+$ countertransport has been demonstrated in erythrocytes from patients with essential hypertension both in studies from North America and France, but not in those from Germany.$^4$ A Danish study found increased erythrocyte $\text{Li}^+ - \text{Na}^+$ countertransport in male but not female patients with essential hypertension.$^5$

Evidence for an altered ($\text{Na}^+ + \text{K}^+$) cotransport is also conflicting. Garay and his colleagues$^6$ reported a 75% reduction in the furosemide-sensitive $\text{Na}^+$ or $\text{K}^+$ fluxes in red cells of patients living in Paris with essential hypertension, while three other groups found marginal or no differences between normal subjects and patients with essential hypertension.$^9$ 12 13

In this study, erythrocyte ($\text{Na}^+ + \text{K}^+$) cotransport and $\text{Li}^+ - \text{Na}^+$ countertransport have been studied in a group of patients under the age of 50 years with a diastolic blood pressure greater than 100 mm Hg and with a family history of hypertension.

Methods

Patient Data

Sodium-lithium countertransport was measured in 27 patients (20 men and seven women) with essential hypertension, ranging in age from 21 to 50 years (mean, 35.4 years). Cotransport was studied simultaneously in 21 of these patients (16 males and five females) who ranged in age from 21 to 50 years (mean, 34.6 years). All patients were Caucasian. Fifth phase diastolic blood pressure, measured in the recumbent
position, equalled or exceeded 100 mm Hg on at least two occasions in all patients studied. No patient had cardiac or renal failure, or papilledema. We studied only hypertensive patients with a positive family history, defined as a history of hypertension in one parent or sibling that required treatment. The diagnosis of essential hypertension was based on clinical findings and on normal results of plasma electrolytes and renal function, microscopic examination of a midstream urine, intravenous pyleography or isotope renal scanning (16 patients), plasma renin studies (15 patients), and urinary catecholamines (13 patients).

The countertransport group included 15 patients on antihypertensive medication (14 on thiazides, four on a beta-blocker, four on prazosin, three on labetolol, three on clopamide, two on amiloride, and one each on methyldopa or clonidine). The cotransport group included 12 patients on a similar range of antihypertensive medications. The remaining patients had never received antihypertensive drugs, and with the exception of one patient who used a salbutamol inhaler for mild asthma, no patient was taking other medications. With the exception of two females (weights: 104 and 84 kg), no patient was obese. All patients were allowed unrestricted dietary salt.

Countertransport was studied in 20 normotensive subjects (17 men and three women) and cotransport in 20 normotensive subjects (16 men and four women). Ages ranged from 22 to 47 years in both groups; the mean age of the countertransport group was 31.7 years and of the cotransport group, 31.8 years. All had blood pressures below 140/90 mm Hg and gave no past or family history of hypertension. None were suffering from a known disease, and none were taking medications or were on dietary restrictions.

### Preparation of Red Cells
Venous blood was collected into heparin; the plasma was removed and then immediately washed three times at room temperature to remove the buffy coat. The washing medium for countertransport studies was either 150 mM LiCl, 20 mM imidazole Cl, pH 7.4, or 150 mM NaCl, and for cotransport studies was 20 mM imidazole Cl, pH 7.4.

### Lithium-Sodium Countertransport
Red cells were loaded with Li⁺ by incubation at a hematocrit of 5% in 150 mM LiCl, 20 mM imidazole Cl, 10 mM glucose, pH 7.4, for 3 hours at 37° C. Extracellular Li⁺ was removed by rapidly washing the cells in ice cold 150 mM KCl, 20 mM imidazole Cl, pH 7.4. Cell cation composition after Li⁺ loading was not significantly different in normotensive subjects or patients with essential hypertension (Table 1). Intracellular Li⁺ (6.0 to 6.6 μEq/ml cells) was above the K⁺ values reported for Li-Na countertransport. Lithium efflux was measured into a Na⁺-rich medium (150 mM NaCl) and a Na⁺-free medium (150 mM KCl) similar to that used by Ibsen et al. The two media were buffered with 20 mM imidazole Cl, pH 7.4, and contained 10 mM glucose plus 0.1 mM ouabain. The KCl medium only contained between 3 and 8 μM Na⁺ and was considered Na⁺-free. Efflux measurements were started; 1.2 ml of packed cells were added to 12 ml of each medium prewarmed to 37° C. Both suspensions were sampled after 15 and 30 minutes of incubation and were rapidly centrifuged; supernatants were removed for Li⁺ determination. The maximal rate of Li⁺-Na⁺ countertransport was taken as the difference between mean Li⁺ efflux rates into Na⁺-rich and Na⁺-free media.

### Potassium and Rubidium Influx
Unidirectional K⁺ or Rb⁺ influx was measured from the uptake of radioactivity by cells incubated in media containing ⁴²KCl (Atomic Energy Commission, Lucas Heights, Australia) or ⁸⁶RbCl (Radiochemical Centre, Amersham, England). Washed red cells were added to give a hematocrit of 10% in prewarmed saline media containing 1, 2, 4, 6, and 10 mM ⁴²KCl (2 μCi/ml) or ⁸⁶RbCl (5 μCi/ml), either in the absence or the presence of furosemide (1.0 mM). All media contained 140 to 150 mM NaCl, 20 mM imidazole Cl, pH 7.4, 10 mM glucose and 0.1 mM ouabain. The latter agent was used to block cation influx via the active pump. Duplicate suspensions were incubated with shaking for 60 minutes at 37° C, and the cells were rapidly washed four times in ice-cold saline to remove extracellular radioactivity. The pelleted cells were then hemolyzed.

### Table 1. Mean Erythrocyte Cation Concentrations

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Na⁺ (μEq/ml cells)</th>
<th>K⁺ (μEq/ml cells)</th>
<th>Li⁺ (μEq/ml cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh erythrocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive (n = 17)</td>
<td>5.7 ± 1.0</td>
<td>92.2 ± 6.4</td>
<td>—</td>
</tr>
<tr>
<td>Essential hypertensive (n = 25)</td>
<td>6.0 ± 1.6</td>
<td>89.3 ± 6.0</td>
<td>—</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>Lithium-loaded erythrocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive (n = 20)</td>
<td>2.5 ± 1.0</td>
<td>88.9 ± 5.4</td>
<td>6.0 ± 1.6</td>
</tr>
<tr>
<td>Essential hypertensive (n = 27)</td>
<td>2.6 ± 1.0</td>
<td>86.8 ± 6.5</td>
<td>6.6 ± 1.0</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
in 0.01 N NH₄OH and counted for radioactivity; the hemoglobin was measured as the cyanmethemoglobin derivative. Cation uptake was calculated, and the cotransport component of the K⁺ or Rb⁺ uptake was taken as the decrement produced by furosemide. Both total and furosemide-sensitive uptakes of Rb⁺ or K⁺ showed a linear dependence on time during the 60-minute incubation time. Uptake was, thus, equated to the influx (V) of the cation. The mean Km and Vmax for cotransport were calculated from the plots of V versus V/S and S/V versus S. Passive leak permeability to Rb⁺ was measured by the influx of the ion in the presence of ouabain plus furosemide and expressed as influx per millimole of Rb⁺ in the medium.

**Outward Na⁺ Cotransport**

Cotransport was determined by quantitating the loss of intracellular Na⁺ from cells incubated in medium of the following composition: 20 mM NaCl, 130 mM choline Cl, 20 mM imidazole Cl (pH 7.4), 10 mM glucose, and 0.1 mM ouabain. The initial concentration of Na in the cells was determined by flame photometry. The cells were then incubated at a hematocrit of 0.6% for 19 hours in this K⁺-free medium at 37°C in the presence or absence of 1 mM furosemide. Intracellular cations were analyzed, and outward Na⁺ cotransport was taken as the difference in cell Na⁺ concentration after 19 hours with and without the diuretic.

**Cell Cation Concentration**

Cells were rapidly washed four times in ice-cold 110 mM MgCl₂, the cell pellets were hemolyzed in 0.01 N NH₄OH plus one drop of 10% tergitol (NP10), and cation concentrations were measured by atomic absorption spectrophotometry with an air-acetylene flame. All samples and standards flamed for K⁺ and Na⁺ contained 27 mM KCl as an ionization suppressant. Samples and standards flamed for K⁺ contained 5 mM CsCl as the ionization suppressant. The standard curve for Li⁺ was linear up to 100 μM and was unaffected by MgCl₂ added in concentrations as high as 75 mM.

**Statistics**

Mean values ± 1 sd are shown in the text; differences between the means were analyzed by Student's t test. Linear correlations of the normotensive and hypertensive groups were compared by analysis of covariance.

**Reproducibility of Transport Measurements**

Repeated measurements of Li⁺-Na⁺ countertransport in erythrocytes from the one subject were highly reproducible when remeasured from 2-week to 2-month intervals, with an average variation of only 0.04 μEq/ml cells/hr (Table 2). Others have also reported an intradividual constancy of countertransport.6-10 Table 2 also shows that the ⁴²Rb cotransport Vmax has a slightly larger average variation between replicates (0.07 μEq/ml cells/hr). This degree of variation is in agreement with another report.⁶

<table>
<thead>
<tr>
<th>Subject</th>
<th>Vmax (μEq/ml cells/hr)</th>
<th>Km (mM)</th>
<th>Vmax (μEq/ml cells/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. McD.</td>
<td>0.54</td>
<td>4.1</td>
<td>0.34</td>
</tr>
<tr>
<td>J.D.S.</td>
<td>0.44</td>
<td>2.4</td>
<td>0.31</td>
</tr>
<tr>
<td>S.H.</td>
<td>0.38</td>
<td>2.3</td>
<td>-</td>
</tr>
<tr>
<td>J.O.</td>
<td>0.54</td>
<td>3.4</td>
<td>0.27</td>
</tr>
<tr>
<td>S.H.</td>
<td>0.75</td>
<td>3.5</td>
<td>0.43</td>
</tr>
<tr>
<td>D.McI.</td>
<td>0.66</td>
<td>4.5</td>
<td>0.52</td>
</tr>
<tr>
<td>P.W.</td>
<td>0.78</td>
<td>3.8</td>
<td>0.34</td>
</tr>
<tr>
<td>J.H.</td>
<td>0.56</td>
<td>4.0</td>
<td>0.35</td>
</tr>
<tr>
<td>R.A.</td>
<td>0.48</td>
<td>4.2</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>0.47</td>
<td>5.0</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Erythrocytes from nine normotensive subjects were assayed concurrently for the kinetic parameters of ⁴²Rb⁻ cotransport and for the maximal rate of Li⁺-Na⁺ countertransport. Each study was repeated on one or two separate occasions spaced 2 weeks to 2 months apart.

**Results**

**Cell Cation Concentrations**

The sodium and potassium concentrations in erythrocytes from normal subjects and patients with hypertension are shown in Table 1. The mean value for cell sodium was slightly greater in the patients than in normal subjects. This difference was not significant (p > 0.10). Similarly, cell potassium in the patients with hypertension was not different from that in normal individuals.

**Cotransport of Potassium or Rubidium**

Unidirectional cation influx was measured on fresh erythrocytes incubated in media containing various concentrations (1 to 10 mM) of ⁴²KCl or ⁴²RbCl. In the presence of ouabain, the influx of either cation showed a hyperbolic dependence on external cation concentration. A major fraction of this influx was blocked by furosemide (Figure 1), and the residual cation influx in the presence of both inhibitors was a linear function of cation concentration. The K⁺ influx was identical to the Rb⁺ influx at each cation concentration (Figure 1). Because of the greater convenience of the ⁴²Rb⁺ isotope, which has a half-life of 19.5 days, the influx of this cation only was measured in the cotransport studies described below.
Kinetic Parameters of Cotransport in Hypertensives

Red cell cotransport parameters were calculated from the furosemide-sensitive component of $^8$Rb influx measured as described above. Two of 21 patients (W.D. and D.R.) had a Vmax for cotransport which was elevated more than twofold above the normal range (Figure 2 left). The mean Vmax for all patients with essential hypertension was 0.60 ± 0.23 μEq/ml cells/hr (n = 21). This value was greater than the mean for the normal subjects, 0.51 ± 0.16 μEq/ml cells/hr (n = 20), although the difference was not statistically significant (p > 0.05). The mean Vmax for the patients was not changed by exclusion of the 12 patients on treatment.

None of the hypertensive patients showed any reduction in the affinity of cotransport when Rb$^+$ was used as the substrate and the Km values always fell within the normal range (Figure 2 center). Moreover, the mean Km for the entire group of hypertensive patients (4.1 ± 1.5 mM) was not significantly different from that of the normal group (4.3 ± 1.9 mM; p > 0.1). Four hypertensive patients showed an increase in passive "leak" permeability to Rb$^+$ ions determined in the presence of ouabain plus furosemide. However, in only two (E.W. and S.T.) was the leak greater than twice the normal mean. The other 17 hypertensive patients all showed normal leak permeability to this cation (Figure 2 right). Despite the high passive permeability to Rb$^+$ ions in erythrocytes from E.W., his cell Na$^+$ concentration (8.0 μEq/ml cells) and Li$^+$ efflux into Na$^+$-rich and Na$^+$-free media were unremarkable. Likewise, erythrocytes from S.T. showed cell Na$^+$ (4.4 μEq/ml cells) and Li$^+$ flux values that were not above the normal range.

Figure 1. Dependence of $^{42}$K$^+$ influx (●) or $^8$Rb$^+$ influx (▲) on external K$^+$ or Rb$^+$ concentration. Red cells from three normal subjects were incubated for 60 minutes at 37°C in the presence of 0.1 mM ouabain either with or without 1.0 mM furosemide. The standard error of the mean is shown by the bars. Analysis of the furosemide-sensitive component gives the maximal velocity and affinity of cotransport (0.45 μEq/ml cell/hr and 2.9 mM, respectively), while the slope of the influx in the presence of ouabain plus furosemide gives the passive "leak" flux.

Figure 2. Left: Maximal rate of inward cotransport of Rb$^+$ in erythrocytes of normal subjects (NT) and of patients with essential hypertension. Center: The affinity of the inward cotransport system for Rb$^+$ substrate expressed as Km. A high value for Km implies low affinity for substrate. Right: Passive "leak" influx of Rb$^+$ substrate expressed as the slope of the regression line between influx and cation concentration for cells incubated with ouabain plus furosemide. Some patients were on medication (○), but most were not (●). Heavy bars represent the means while lighter bars show ± 1 sd.
Correlation of Inward and Outward Cotransport

Although inward and outward cotransport are closely coupled in normal red cells, it is possible that outward but not inward cotransport is impaired in the erythrocytes of patients with essential hypertension. Outward cotransport was assayed by the furosemide-sensitive movement of Na ions up its concentration gradient induced by net K efflux from fresh erythrocytes incubated in K-free media. The initial cell Na+ of 6.9 ± 1.4 and 7.7 ± 0.8 μEq/ml cells in the normotensives (n = 11) and hypertensives (n = 9) groups, respectively, decreased after incubation in the K-free media. However, this decrease was prevented by furosemide (Table 3). The magnitude of this uphill cotransport of Na+ was defined by the furosemide-sensitive component (0.9 to 4.1 μEq/ml cells/19 hr) and was less than the inward cotransport assayed in parallel by unidirectional fluxes (Table 3). There was no significant difference in the mean outward Na+ cotransport between normotensive subjects and hypertensive patients (p = 0.3; Table 3). In all 20 subjects shown in Table 3, a parallel measurement of erythrocyte 86Rb+ influx was performed in the presence and absence of furosemide. In the hypertensive patients, a positive correlation was observed between Vmax for inward cotransport, measured by 86Rb+ influx, and furosemide-sensitive net outward cotransport of Na+ (n = 9; r = 0.77; p < 0.01). This comparison of inward and outward cotransport is shown in Figure 3. Analysis of covariance showed no significant differences between the hypertensive (n = 9) and normotensive (n = 11) groups (0.02 < p < 0.05). Figure 3, therefore, shows the positive correlation obtained between inward and outward cotransport in the red cells of all normal subjects and hypertensive patients studied (r = 0.73; p < 0.005).

Lithium Efflux into Various Media

To study the ionic conditions that define Li+-Na+ countertransport, lithium efflux from Li+-loaded cells was measured into various media. Li+ efflux was greater into isotonic Na+ media than into isotonic K+ media or MgCl2 (Table 4). An increment in Li+ efflux due to extracellular Na+ was always observed and defines the magnitude of the Li+-Na+ countertransport component. Extracellular MgCl2, however, produced a variable effect on Li+ efflux. In one individual, MgCl2 (5 or 75 mM) decreased Li+ efflux. This effect was independent of Li+-Na+ countertransport since the Mg2+-induced decrement occurred in both Na+-rich and Na+-free media (Subject P. McD., Table 4). These results suggested that extracellular Mg2+ inhibited the passive downhill Li+ leak. In another individual, extracellular MgCl, had little or no effect on Li+ efflux (Subject L.B., Table 4). In every case, the magnitude of countertransport could be defined as the difference in Li+ efflux between NaCl + 5 mM MgCl2 and KCl + 5 mM MgCl2.

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Correlation of inward and outward cotransport in patients with essential hypertension (●) (n = 9) and normotensive (○) subjects (n = 11). Inward cotransport was measured by the maximal velocity (Vmax) of furosemide-sensitive Rb+ influx, while outward cotransport was measured by furosemide-sensitive net Na+ loss from cells incubated in K+-free media.

**Table 3.** Net Outward Cotransport of Na+ Against a Na+ Gradient for Hypertensive and Normotensive Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>Inhibitor</th>
<th>Cell Na+ (μEq/ml cells)</th>
<th>Furosemide-sensitive net Na+ efflux (μEq/ml cells/19 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial 19 hr</td>
<td></td>
</tr>
<tr>
<td>Normotensives</td>
<td>11</td>
<td>Ouabain</td>
<td>4.7 ± 0.2</td>
<td>6.9 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ouabain + furosemide</td>
<td>7.2 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Essential hypertensives</td>
<td>9</td>
<td>Ouabain</td>
<td>4.8 ± 0.9</td>
<td>7.7 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ouabain + furosemide</td>
<td>7.5 ± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

Erythrocytes were incubated at a hematocrit of 0.6% in medium composed of 20 mM NaCl, 130 mM choline Cl, 20 mM imidazole Cl, 10 mM glucose, and 0.1 mM ouabain either in the presence or absence of 1 mM furosemide. Outward Na+ cotransport was taken as the difference in cell Na+ concentration after 19 hours with and without furosemide. Values are means ± 1 SD.

**TABLE 4.** Lithium Efflux into Various Media

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Method</th>
<th>Initial 19 hr, μEq/ml cells</th>
<th>Li+ efflux, μEq/ml cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>NaCl + 5 mM MgCl2</td>
<td>4.7 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>KCl + 5 mM MgCl2</td>
<td>7.2 ± 1.2</td>
<td>6.5 ± 1.0</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>NaCl + 5 mM MgCl2</td>
<td>5.6 ± 0.8</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>KCl + 5 mM MgCl2</td>
<td>8.1 ± 1.4</td>
<td>7.3 ± 1.2</td>
</tr>
</tbody>
</table>

Rb* influx (Table 3).
Both determinations gave the same result (Table 4). In this study, $\text{Li}^+\text{-Na}^+$ countertransport was taken as the difference in $\text{Li}^+$ efflux into isotonic NaCl and KCl media respectively, as described in a recent report.\textsuperscript{10}

### Lithium-Sodium Countertransport

Cells were loaded with lithium, and its efflux was measured into both isotonic NaCl and KCl media. The difference was taken as the maximal $\text{Li}^+\text{-Na}^+$ countertransport. The mean countertransport rate for normotensive subjects was $0.32 \pm 0.09 \mu\text{Eq/mL cells/hr}$ ($n = 20$). This rate did not differ significantly from that of patients with essential hypertension ($0.35 \pm 0.16 \mu\text{Eq/mL cells/hr}$, $n = 27$) (Figure 4). However, two male patients (W.D. and D.R.) showed markedly elevated values for red cell countertransport. These values were more than twofold greater than the mean for the normotensive group. Both patients also showed a cotransport Vmax above the normal range although their linear cation leak permeability, judged by $\text{Rb}^+$ influx in the presence of ouabain plus furosemide, was not different from that of normal subjects.

### Relationship of Countertransport to Cotransport

Figure 5 shows the lack of correlation observed between $\text{Li}^+\text{-Na}^+$ countertransport and $(\text{Na}^+ + \text{K}^+)$ cotransport in erythrocytes from all hypertensive patients and normotensive subjects studied ($n = 36$, $r = 0.31$, $p > 0.05$).
Discussion

The major finding of this study is that the erythrocytes of most patients with untreated essential hypertension show normal values for cotransport of Rb$^+$ and Na$^+$ as well as for normal countertransport of Li$^+$ for Na$^+$. Our study also demonstrates that Rb$^+$ is a faithful analog of K$^+$ in erythrocyte transport systems. Our kinetic parameters for cotransport of $^{86}$Rb$^+$ as substrate, therefore, can be directly equated with values obtained with the physiological substrate, K$^+$ ions. The criteria used in our selection of patients included ages between 20 and 50 years, diastolic pressures over 100 mm Hg, and a positive family history of hypertension. These strict diagnostic criteria and the use of isotopic methods give weight to the conclusion that erythrocyte cotransport and countertransport are usually normal in patients with essential hypertension. Two of the 21 patients studied, however, demonstrated a marked increase in (Na$^+ + K^+$) cotransport as well as elevated Li$^+$-Na$^+$ countertransport measured at maximal velocities. Two other patients demonstrated a marked increase in the erythrocyte "residual leak" permeability to Rb$^+$ with values twofold greater than those observed for other patients and normal individuals (Figure 2 right). None of the patients showed either increased or reduced affinity of the cotransport system for Rb$^+$ ions. It is unlikely, therefore, that abnormal affinity of inward cotransport for its cosubstrate is a feature of untreated essential hypertensives (Figure 2 center). In contrast, the outward cotransport system in erythrocytes has been reported to show low affinity for Na$^+$ ions in some patients with essential hypertension. Our study shows heterogeneity in cation permeability of erythrocytes in essential hypertension. One etiology of essential hypertension may be an altered transport of Na$^+$ at the level of the cell membrane. The finding of two patients in our study with raised (Na$^+ + K^+$) cotransport as well as high Li$^+$-Na$^+$ countertransport offers support for this possibility. However, only a small subset of patients in our study demonstrates this abnormal cation transport.

The trend to an increased erythrocyte Na$^+$ concentration in essential hypertension (Table 1) has been noted by other investigators, although in this and other studies the increment did not reach statistical significance. Only three studies have reported a statistically significant increase in erythrocyte Na$^+$ concentration in patients with essential hypertension, although the very high value obtained for normal subjects in Nigeria emphasizes the importance of comparing groups of similar racial background. Indeed, the increased erythrocyte Na$^+$ concentration noted in one study of Canadian patients with essential hypertension was not statistically significant when non-Caucasian patients were excluded from the study. This latter study also found an increased net Na$^+$ influx, while a second Canadian study found increased Na$^+$ efflux from erythrocytes of patients with essential hypertension. In neither study was it clear, however, whether passive leak permeability or Na$^+$ cotransport components were increased.

The initial concept of erythrocyte cotransport was based on the isotopic influxes of K$^+$ and Na$^+$ ions, an approach that yields values for both Vmax and Km of a furosemide-sensitive transport system. Subsequent reports of erythrocyte cotransport in essential hypertension have usually measured net cation movements, either as net outward Na$^+$ and K$^+$ losses or as net Rb$^+$ uptake, but none of these net movements can give the Vmax and Km of this transport system. In these three studies, as well as in a fourth in which the Na$^+$ influx rate constant alone was measured, the furosemide-sensitive cation movements were uniformly normal in patients with essential hypertension. In contrast, cotransport has been reported to be decreased in hypertensive patients from Paris. Only the present study has defined erythrocyte cotransport by isotopic influxes as used in the initial description of this transport system. Moreover, only patients with a family history of hypertension were included in our study, which ensured a strong genetic component in the etiology of their hypertension. Our present results fail to confirm Garay et al.'s conclusion that cotransport has reduced velocity in essential hypertension. These authors only measured outward cotransport of Na$^+$ from cells loaded with Na$^+$ by the p-chloromercuribenzenesulfonate (PCMBs) technique, after which the cells were incubated in K$^+$-free media containing NaCl at a concentration slightly greater than the intracellular concentration. Our present study found that cotransport was normal in most and even increased in two patients when measured either in the inward direction with $^{86}$Rb$^+$ influx (Figure 2) or as outward net Na$^+$ cotransport into K$^+$-free media against a slight Na$^+$ gradient (Table 3).

One possible source of the discrepancy between the two studies may be the pretreatment of red cells with PCMBs used by Garay et al. to alter cell cation content, a procedure that may have changed both the cell volume and the cotransport fluxes of cations. The data in Figure 3 exclude any major vectorial dissociation in the erythrocyte cotransport system since analysis of covariance showed no significant differences in the hypertensive and normotensive groups. However, because of the scatter in data points in Figure 3, it is not possible to exclude the possibility that some hypertensive patients had a reduced affinity of cotransport for internal Na$^+$ ions.

Our present report also fails to confirm the increase in countertransport described in a majority of patients with essential hypertension of Caucasian origin. However, two of our patients did show erythrocyte transport alterations similar to those described by...
Adragna et al. in that both erythrocyte Li⁺-Na⁺ countertransport and (K⁺ + Na⁺) cotransport were increased above the normal range. Reports from Boston²,⁶ indicate that two-thirds of patients with "established" essential hypertension have a countertransport value that is elevated above the normal range. In contrast, our study suggests that only 10% have elevated values. The Boston group finds that patients with mild or labile hypertension have a normal erythrocyte countertransport and suggests that discrepancies such as those above may relate to the inclusion of mild disease in other studies.⁶ Patients with mild or labile hypertension were excluded from our study by strict entry criteria. Other investigators have also found that the majority of their hypertensive patients have an erythrocyte countertransport system that lies within the normal range.⁹,¹⁰,²⁴,²⁵

Recent data from the Paris group indicate that three of 28 hypertensive patients have clearly elevated erythrocyte cotransport and countertransport systems,¹⁷ which suggests an incidence of this dual abnormality (12%) that is in line with our value. Differences among studies may result from the interaction of genetic, endocrine, and age-related factors that may affect red cell cation permeability. The Vmax of the Li⁺-Na⁺ countertransport system is higher in normotensive males than in females¹⁰,²⁴,²⁶ and higher in normotensive adults than in youths.²⁴ Values for the Vmax of the Li⁺-Na⁺ countertransport system have ranged from a mean of 0.19 and 0.25 μEq/ml cells/hr in two studies from Italy,²⁵,²⁷ 0.27 μEq/ml cells/hr in a study from Utah,²⁴ 0.29 μEq/ml cells/hr in Boston,⁶ 0.32 μEq/ml cells/hr in Melbourne (this study), to 0.38 μEq/ml cells/hr in Copenhagen.¹⁰

It has recently become clear that endocrine as well as genetic factors may affect Li⁺-Na⁺ countertransport, since the activity of this transport system rises substantially in both normotensive and hypertensive pregnant women and reaches a maximum late in the third trimester.²⁸ Sex differences have been shown in the Copenhagen study¹⁰ of Li⁺-Na⁺ countertransport in essential hypertension. Although approximately 30% of hypertensive males demonstrated elevated countertransport, the values in hypertensive females were uniformly normal. It is of interest that the two patients with elevated Li⁺-Na⁺ countertransport in our study were both males. Obesity, as measured by body mass index, has been correlated with the magnitude of erythrocyte countertransport in male hypertensives.²⁹ None of the males in our study were morbidly obese, and the two obese females had normal values for Li⁺-Na⁺ countertransport.

Technical factors may also affect the measurement of countertransport. Quantitation of Li⁺-Na⁺ countertransport is based on the difference between Li⁺ efflux from Li⁺-loaded cells incubated in "high Na⁺" and "zero-Na⁺" media, the latter being either isotonic MgCl₂-sucrose⁴ or KCl media.¹⁰ Table 4 shows that in some individuals Mg²⁺ ions may affect Li⁺ efflux through a pathway that is separate and distinct to Li⁺-Na⁺ countertransport fluxes. Erythrocyte Li⁺ efflux is unaffected by Mg²⁺ ions in other individuals, however, and this interindividual variation may explain why two studies failed to find differences in Li⁺ efflux into a variety of zero-Na⁺ media (MgCl₂, choline Cl, KCl),¹⁰,³⁰ Our present study emphasizes that countertransport must be assayed by parallel incubation of cells in "high Na⁺" and "zero-Na⁺" media where the paired media are identical with respect to the presence or absence of MgCl₂. Unlike the Boston and Italian studies,⁶,³¹ we could not demonstrate a correlation between the Vmax for countertransport and cotransport when the hypertensive and normotensive groups were considered together (Figure 5). The correlation reported⁶,³¹ rested largely on the inclusion of hypertensives with both elevated cotransport and countertransport. The low prevalence of this combined abnormality in our study accounts for the discrepancy. Our results emphasize that there is no specific linkage between the two transport pathways.

Greater variability existed for the Vmax of erythrocyte cotransport than for countertransport in the same group of normal subjects (Table 2). Normal subjects in our Melbourne study gave a lower cotransport Vmax for K⁺ (or Rb⁺) than a normal group studied by us in Philadelphia¹⁶ by a similar technique (mean Vmax, 0.51 and 0.82 μEq/ml cells/hr, respectively). One source of variability in cotransport measurements may relate to the nonspecificity of furosemide as an inhibitor of cotransport. In particular, furosemide will inhibit K⁺-K⁺ exchange diffusion in Na⁺-free media.² This may be attributed in part to inhibition of K⁺ influx via a K⁺-Cl⁻ cotransport system, which has recently been demonstrated in sheep erythrocytes.³² Our estimates of the magnitude of K⁺-Cl⁻ cotransport in human erythrocytes suggest that it comprises only some 10% of the furosemide-sensitive fluxes, so that the latter gives a good estimate of the Na⁺-K⁺ cotransport pathway.

A second source may be the cholesterol loading of low density lipoproteins that regulate the cholesterol/phospholipid ratio of the red cell membrane. This parameter in turn has been shown to affect the Vmax of the cotransport system for its two cosubstrates, Na⁺ and K⁺ ions.¹⁰ Environmental factors such as membrane cholesterol may also affect erythrocyte countertransport. Longitudinal studies on the same individuals under different dietary and environmental conditions will be necessary to fully identify factors that may alter erythrocyte cation fluxes.

It is likely that environment as well as genetic factors do play some part in the magnitude of increased erythrocyte cotransport and countertransport. This is highlighted by the recent report³³ that erythrocyte Li⁺-Na⁺ countertransport is dependent on a dialyzable plasma factor, the removal of which acutely reduces lithium fluxes. Other studies,³,⁷ however, have demonstrated a genetic influence by finding that increased countertransport in patients with essential hypertension has been inherited by their normotensive offspring. Moreover, two studies of cotransport in identical twins showed close concordance between the twin pairs in their Na⁺ cotransport fluxes.¹³,²⁴
The results in this and other studies suggest that essential hypertension is heterogeneous in etiology. One subgroup of patients is characterized by increased passive leak of Rb+ ions into erythrocytes. Another subgroup is characterized by high erythrocyte cotransport and countertransport, and this abnormality has varying prevalence in different countries.

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