Mechanism of Alteration of Sodium Potassium Pump of Erythrocytes from Patients with Chronic Renal Failure

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Abstract. We examined intracellular electrolytes, K influx, and [^{3}H]ouabain-binding capacity of erythrocytes from 32 normal subjects and 45 patients with end-stage renal failure on dialysis, including 16 with high intracellular Na (mean 17.3±3.9 mmol/liter cell water). The [^{3}H]ouabain-binding capacity of erythrocytes with high cell Na was markedly reduced as compared with that of erythrocytes from normal subjects (274±52 vs. 455±59 sites/cell, $P < 0.001$). The mean serum creatinine was higher in the uremic group with high cell Na. There was a significant linear correlation between intracellular Na and [^{3}H]ouabain-binding in both normal and uremic subjects. Cross-incubation of normal cells with uremic plasma for 24 h failed to reduce [^{3}H]ouabain-binding capacity of normal cells. In spite of a substantial increase in cell Na, K pump influx was not higher in uremic erythrocytes with high cell Na. When intracellular Na was altered with nystatin (cell Na equal to 120 mmol/liter cell water in both groups), K pump influx was proportional to the number of Na-K pump sites so that the ion turnover rate per pump site was similar in the two groups. Uremic plasma failed to depress K pump influx of normal erythrocytes. The passive net influx of Na in uremic cells with high intracellular Na was not different from that observed in erythrocytes from normal subjects. When erythrocytes were separated by age on Percoll density gradients, the number of Na-K pump sites of the youngest uremic cells was significantly lower than that of the youngest normal cells, suggesting that decreased synthesis of Na-K pump sites, rather than accelerated loss of Na-K pump sites during aging, was responsible for the decrease in [^{3}H]ouabain-binding capacity of erythrocytes from uremic subjects. Taken together, these findings suggest that a decrease in the number of Na-K pump sites plays a major role in the abnormality of Na-K pump of erythrocytes from patients with chronic renal failure.

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Introduction

The concentration of Na inside the cell and the transport of Na and K across cell membranes are largely mediated by a specific transport system, consisting of a Mg-dependent Na-K-ATPase and termed the Na-K pump (1–4). An increase in intracellular Na was first described in erythrocytes from some patients with chronic renal failure some two decades ago (5). Subsequently, several groups of investigators have provided evidence for a defect in Na transport of erythrocytes (6–9), leukocytes (10), and skeletal muscle (11) from uremic subjects.

Earlier efforts to explore the mechanism of the rise in intracellular Na were limited to measurements of pump-mediated Na efflux, the passive Na leak, and Na-K-ATPase activity (5–12). In the present study, the number of Na-K pump sites was quantitated by the technique of [^{3}H]ouabain binding (13–23). The accurate quantitation of the number of pump sites in patients with normal and high cell Na permitted an examination of the role of alteration in the number of ion pump units in raising intracellular Na. The role of alteration of ion turnover rate and passive Na permeability in raising cell Na was also examined. In addition, the capacity of uremic plasma to alter the [^{3}H]ouabain-binding capacity and K pump influx of normal erythrocytes was also investigated. The results of our investigation suggest that a decrease in the number of Na-K pump sites plays a pivotal role in the defect of Na transport of erythrocytes from patients with chronic renal failure.

Methods

Subjects

Subjects were divided into two groups: (*a*) Group I: Normal group. This group consisted of 32 adults, mean age 37 yr. (*b*) Group II: End-

stage renal failure. This group consisted of 45 patients on chronic maintenance dialysis, mean age 58 yr. All patients were stable, ambulatory, and free from intercurrent illness. Patients taking cardiac glycosides, diuretics or prednisone, or transfused within 6 wk of the study were excluded. Serum cortisol, triiodothyroxine (T_3), and thyroxin (T_4) were normal in patients with chronic renal failure.

Preparation of erythrocyte suspension

Fresh heparinized blood was drawn predialysis and centrifuged within 1 h of collection. The plasma, the buffy coat, and the topmost layer (10%) of the erythrocytes were discarded and the remaining erythrocytes were suspended in ice-cold isotonic $MgCl_2$. During each of the three washes that followed, the supernatant and the topmost layer of erythrocytes were discarded. The precise hematocrit of the final washed erythrocyte suspension was determined in triplicate.

Intracellular electrolytes

Intracellular Na and K were measured by flame photometry from lysates of washed cells (23). The volume fraction of water was estimated from dry and wet weight of cell suspensions, density of cells, and the hematocrit as described previously (23).

[^{3}H]Ouabain-binding assay

The techniques for measuring specific activity of [^{3}H]ouabain, for ascertaining that the radioactivity was attached to the ouabain molecule, and for measuring nonspecific ouabain binding have been described before (23).

[^{3}H]ouabain binding was measured by the method of Joiner and Lauf (18). Cells were incubated for 180 min with 100 nM [^{3}H]ouabain (sp. act., 19.5–14.2 Ci/mmol) in a medium containing, in millimolar, NaCl, 130; sucrose, 20; Tris–HCl, 10; and glucose, 10; pH 7.4, at 37°C. After incubation, cells were separated from unbound [^{3}H]ouabain by density gradient, lysed, and washed twice to remove hemoglobin. Cell membranes were then solubilized by 0.1 N NaOH, treated with HCl to reduce chemiluminescence, and counted in a scintillation spectrometer. [^{3}H]ouabain-binding sites per cell were calculated as described previously (23).

Effects of uremic plasma on [^{3}H]ouabain-binding capacity

Freshly harvested erythrocytes from normal subjects were incubated at 37°C for 24 h in (*a*) their own plasma, (*b*) heterologous normal plasma, (*c*) plasma from chronic renal failure patients with normal ouabain binding, and (*d*) plasma from chronic renal failure patients with reduced ouabain binding. Care was taken to ensure ABO and Rh blood group compatibility. After this incubation, erythrocytes were washed and processed for [^{3}H]ouabain binding as described above.

K influx

Fresh, unaltered cells. K influx was measured by the method of Sachs and Welt (24). Washed cells (2–4% hematocrit) were suspended at 37°C in a medium containing, in millimolar, NaCl, 140; KCl, 10; Tris–HCl, 10; glucose, 10; pH 7.4, at 37°C in the presence and absence of 0.5 mM ouabain. The isotope ^{42}KCl was added at zero time. Aliquots of cell suspensions were taken at 1 h, washed three times with chilled $MgCl_2$, and the radioactivity of the washed cell pellet was counted in a gamma counter (Gamma 4000, Beckman Instruments, Fullerton, CA). Pump-mediated uptake was calculated as the difference in uptake of paired samples with and without 0.5 mM ouabain (15).

Cells with altered intracellular cation concentrations. Studies were performed on erythrocytes from seven normal subjects and five uremic subjects with high intracellular Na. Cell Na and K were altered by the nystatin methods of Cass and Dalmark (25), modified by Joiner and Lauf (20). Washed cells (0.2–0.4 ml) were suspended at 4°C for 0.5 h in 10 ml of medium containing (in millimolar) NaCl, 120; KCl, 15; tetramethyl ammonium chloride, 55; glucose, 5; and nystatin in dimethyl sulfoxide (80 mg/liter final); pH 7.2, at 4°C. Cells were washed three times with the same medium without nystatin and then four times with isotonic $MgCl_2$, pH 7.4, at 25°C. Alteration of cell volume was <5% and monitored by mean corpuscular hemoglobin concentration (26). Cell Na and K concentration and hematocrit were measured and K influx was measured as described above, except for the substitution of ^{86}Rb for ^{42}K as the tracer.

Effect of uremic plasma on K influx. Plasma was obtained predialysis from 12 uremic subjects with high cell Na. Normal fresh erythrocytes were washed as described above and suspended at 5% hematocrit in their own plasma or in uremic plasma. The plasma was passed through glass wool to deplete leukocytes and platelets, and fortified with 10 mM glucose and enough KCl to give a final concentration of 10±0.3 mM KCl. The influx was measured by using ^{86}Rb as a K analog, as described above after a preincubation period of 30 min.

Passive permeability

Washed erythrocytes from seven normal subjects and seven uremic subjects with high cell Na were suspended at 10% hematocrit in a medium containing (in millimolar) NaCl, 150; KCl, 5; Tris–HCl, 5; glucose, 10; pH 7.4, at 37°C. The net uptake of cell Na was measured at 37°C in paired tubes with and without 0.5 mM ouabain (27). Aliquots of cell suspension were obtained at 0, 2, and 4 h, washed four times in $MgCl_2$, and the intracellular Na and K were measured as described above.

Separation of erythrocytes by age on Percoll gradients

Erythrocytes were separated by age on a self-generated continuous density gradient of Percoll. Percoll was diluted to a density of 1.1 kg/liter with phosphate-buffered saline and spun at 5600 *g* for 90 min in a fixed angle rotor at 20°C. The gradient so obtained was monitored by Sephadex density marker beads and provided a wide separation between the densities of 1.085 and 1.100 kg/liter. Washed erythrocytes, depleted the leukocytes by passage through a cellulose column (28) and adjusted to a hematocrit of 20±1% with phosphate-buffered saline, were layered over the preformed Percoll gradient and spun for 15 min at 800 *g*. The erythrocytes were aspirated with the aid of a peristaltic pump and washed free of Percoll by three washes with the same medium that was used for the [^{3}H]ouabain-binding assay. The cells were processed for [^{3}H]ouabain-binding assay as described above. Erythrocyte glucose 6 phosphate dehydrogenase (G_6PD), an accepted marker for cell age in normal subjects (29, 30) was measured by the change in optical density at 340 mM due to nicotinamide-adenine dinucleotide phosphate reduction using the Sigma test kit (Sigma Chemical Co., St. Louis, MO). The validity of this enzyme as a marker for cell age in uremia was established by the observation that the plot of cell density vs. G_6PD levels yielded similar slopes and intercepts in normal and uremic groups.

Statistics

Results were expressed as mean±one SD. Statistical analyses were performed using unpaired *t* test, unless stated otherwise. Differences

1. *Abbreviations used in this paper:* CRF, chronic renal failure; G_6PD, glucose 6 phosphate dehydrogenase.

between slopes of regression lines were computed as described by Snedecor and Cochran (31).

Solutions and chemicals

The radioisotopes [^{3}H]ouabain and ^{42}H were obtained from New England Nuclear, Boston, MA, and ^{86}Rb was from Amersham Corp., Arlington Heights, IL. Percoll and Sephadex density marker beads were purchased from Pharmacia, Inc., Piscataway, NJ. All other chemicals were obtained from Sigma Chemical Co. or VWR Scientific, Inc., Rochester, NY.

Results

Intracellular cation concentrations

The mean intracellular Na concentration, expressed as millimoles per liter cell water, was 9.8±1.6 in 32 normal subjects and 12.5±4.5 in 45 patients with chronic renal failure ($P < 0.01$).

To examine the abnormality of the Na-K pump selectively in patients with high intracellular Na concentrations, patients with chronic renal failure were further subdivided into two groups, according to the upper limit of cell Na in our normal subjects, which was 13 mmol/liter cell water. The 29 patients with cell Na < 13 mmol/liter cell water (mean 9.9±2.0) were classified as group II *A* (chronic renal failure, normal cell Na). The 16 patients with cell Na > 13 mmol/liter cell water (mean 17.3±3.9 mmol/liter cell water) were grouped as group II *B* (chronic renal failure, high cell Na) (Fig. 1).

Mean cell K concentration was 139.9±5.8 mmol/liter cell water in the normal group, not significantly different from the two subgroups with chronic renal failure. The volume fraction of water, expressed as liter per liter cells, was 0.697±0.013 in normal subjects, and 0.704±0.015 in patients with chronic renal failure (NS).

[^{3}H]Ouabain binding

Specific [^{3}H]ouabain binding, defined as the binding of [^{3}H]ouabain to erythrocytes which could be displaced by nonradioactive ouabain, accounted for at least 98% of the total [^{3}H]ouabain bound to erythrocytes from both the normal and the uremic subjects.

The specificity and physiologic importance of [^{3}H]ouabain binding to uremic erythrocytes was underscored by our observations that the fractional occupation of ouabain binding in both groups was linearly correlated with the fractional inhibition of active K influx. Thus, after 150 min of incubation of 0.1 mM ouabain, >98% of active K influx was inhibited in both groups, suggesting that our values for [^{3}H]ouabain binding obtained under these conditions represented maximal ouabain-binding capacity in both the normal and the uremic erythrocytes (13, 16, 19, 20, 23).

Specific [^{3}H]ouabain-binding capacity of intact erythrocytes in normal subjects averaged 455±59 sites/cell (8.68 pmol/ml cells), in good agreement with values obtained previously by us (23) and others (18–20). Erythrocytes from 45 patients with chronic renal failure bound significantly less [^{3}H]ouabain, 338±75 sites/cell (6.38 pmol/ml cells), as compared with cells from normal subjects ($P < 0.001$). The reduction in [^{3}H]ouabain-binding capacity of erythrocytes with high cell Na (274±52 sites/cell, 5.23 pmol/ml cells) was even more striking as compared with both normal subjects (455±59 sites/cell, $P < 0.001$) and dialysis patients with normal cell Na (373±61 sites/cell, $P < 0.05$) (Fig. 1).

There was a significant, linear, inverse correlation between [^{3}H]ouabain-binding capacity and cell Na in normal subjects ($r = 0.74$, $P < 0.01$), suggesting that the number of Na-K pump sites per cell plays an important role in determining the cell Na in normal subjects (Fig. 2). An inverse linear correlation

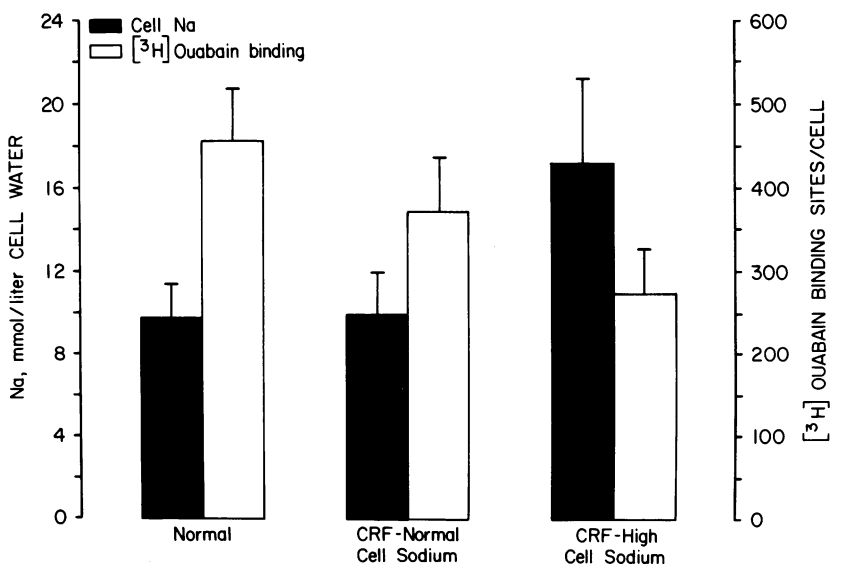

Figure 1. Mean intracellular Na concentrations and maximum [^{3}H]ouabain-binding capacity of erythrocytes from 32 normal subjects, 29 patients with chronic renal failure (CRF) and normal intracellular Na, and 16 patients with chronic renal failure and high intracellular Na.

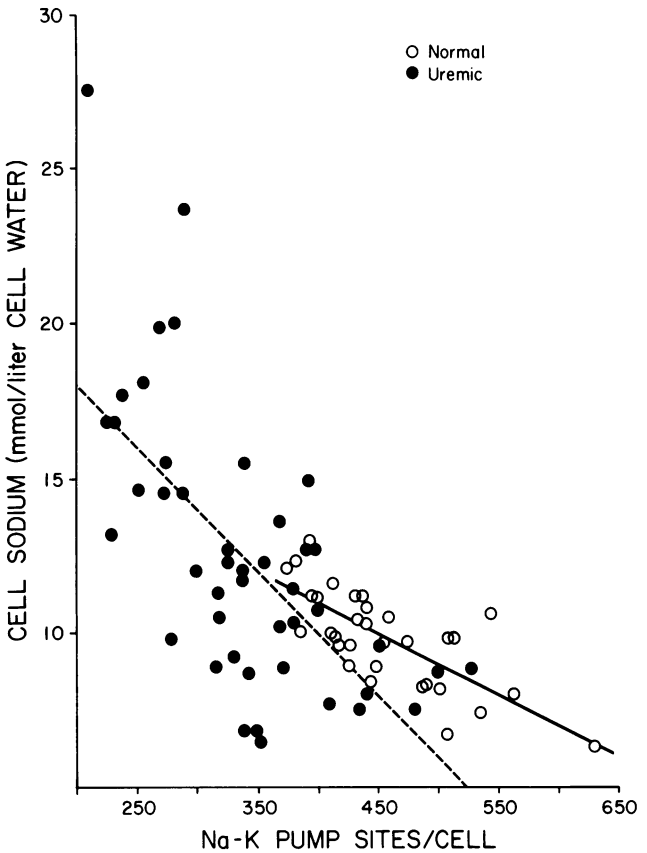

Figure 2. Correlation between maximal [^{3}H]ouabain-binding capacity and intracellular Na concentration in 32 normal subjects and 45 patients with chronic renal failure on dialysis. The regression line for the normal group was $y = 18.74 - 0.02x$, $r = 0.74$, $P < 0.01$ and that for the patient with renal failure was given by $y = 25.98 - 0.04x$, $r = 0.66$, $P < 0.01$. The slopes of the two regression lines were significantly different ($P < 0.05$).

between [^{3}H]ouabain binding and cell Na was also detected in 45 patients with chronic renal failure ($r = 0.66$, $P < 0.01$) (Fig. 2). The slope of the linear regression line in patients with chronic renal failure was significantly different ($P < 0.05$) as compared with the slope obtained in normal subjects (Fig. 2).

Effect of uremic plasma on [^{3}H]ouabain binding

Maximum [^{3}H]ouabain binding was 414±34 sites/cell when normal erythrocytes were incubated for 24 h in their own plasma, 388±53 sites/cell when incubation was performed in plasma from other normal individuals, 380±68 sites/cell when cells were incubated with plasma from chronic renal failure patients with normal cell Na, and 390±63 sites/cell when plasma from patients with chronic renal failure and high cell Na was employed for cross-incubation with normal cells. Thus, we failed to detect evidence for a circulating substance in the plasma of patients with chronic renal failure which could cause interference with the [^{3}H]ouabain-binding assay in those patients.

K influx

Unaltered cells (Table I). Several groups of investigators have shown that an increase in intracellular Na increases K pump influx (20, 32–34). However, in spite of a substantially higher intracellular Na concentration in the uremic erythrocytes, ^{42}K-pump influx was not significantly different in uremic erythrocytes with high cell Na as compared with erythrocytes from normal subjects (0.99±0.15 vs. 1.04±0.09 mmol/liter cells per h, Table I).

K influx with cells of altered cation composition (Table I, Fig. 3). To equalize the intracellular Na concentration between the normal and uremic erythrocytes, the cation composition of erythrocytes was altered with nystatin. K influx was measured at saturating concentrations of intracellular Na (120 mmol/liter cell water) and extracellular K (10 mmol/liter). K pump influx was reduced in uremic erythrocytes as compared with normal cells (1.32±0.28 vs. 2.35±0.39 mmol/liter cells per h, $P < 0.001$). The reduction in K pump influx in uremic erythrocytes was proportional to the reduction in Na-K pump sites (Fig. 3). The K ion turnover per pump site was similar in normal and uremic erythrocytes (5099±283 and 4671±556 K ions/pump site per min). Thus, in spite of a marked reduction in the number of Na-K pump sites, the cation transport capacity of individual pump site from uremic erythrocytes was normal.

K influx in the presence of uremic plasma. Active K influx of normal erythrocytes was 1.09±0.12 mmol/liter cells per h

Table I. Intracellular Electrolytes, Unidirectional Tracer K Fluxes, and Passive Permeability of Intact and Nystatin-treated Erythrocytes

	Normal group	Group II *A*: CRF–Normal cell Na	Group II *B*: CRF–High cell Na
Intact unaltered cells			
Intracellular Na	10.9±1.9	10.7±1.2	16.1±2.0*
Intracellular K	139.9±5.8	142.0±6.0	139.7±6.9
Ouabain-sensitive ^{42}K influx	1.04±0.09	0.88±0.15	0.99±0.15
Ouabain-insensitive ^{42}K influx	0.56±0.14	0.56±0.23	0.41±0.12
Net passive Na influx	1.21±0.19		1.08±0.20
Nystatin-treated cells			
Intracellular Na	119.8±4.5		122.0±3.6
Intracellular K	14.7±1.8		16.9±2.0
K pump influx	2.35±0.39		1.32±0.28*
[^{3}H]ouabain binding (sites per cell)	401±52		243±28*
K ion turnover per pump site per minute	5099±283		4671±556

* $P < 0.001$. Intracellular Na and K are expressed as millimoles/liter cell water. K pump influx and net passive Na influx are expressed as millimoles per liter cells per hour.

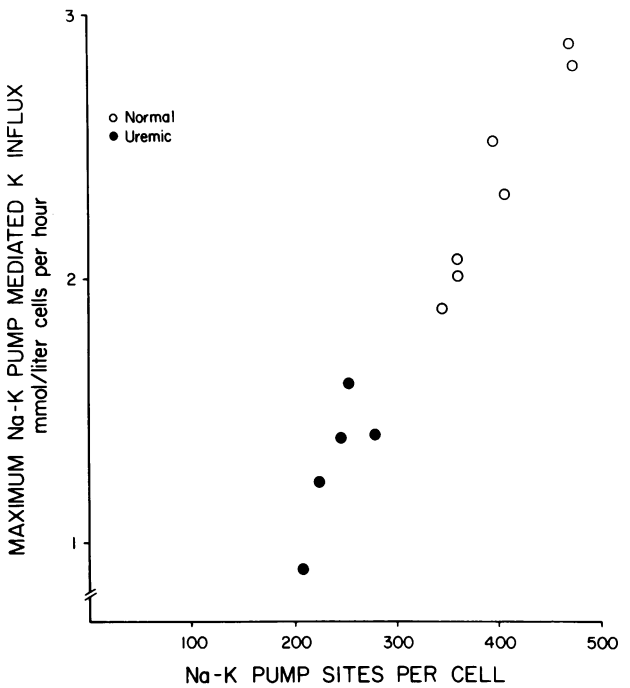

Figure 3. K pump influx of nystatin-treated erythrocytes from seven normal subjects and five dialysis patients with high intracellular Na. After nystatin treatment, the intracellular Na, expressed as millimoles per liter cell water, was 119.8±4.5 for the normal erythrocytes and 122.0±3.6 for the erythrocytes from dialysis patients (NS). Cells were incubated in a medium containing 140 mM NaCl and 10 mM KCl and the flux was measured with ^{86}Rb as the tracer. The regression line is given by the equation $y = 0.007x - 0.324$, $r = 0.964$. Note that the ordinate is broken and that this line passes close to the origin.

in their own plasma and 1.08±0.12 mmol/liter cells per h in plasma of uremic subjects with high cell Na (NS). The ouabain-insensitive influx was also similar (0.56±0.09 vs. 0.52±0.16 mmol/liter cells per h).

Passive permeability

In the absence of ouabain, normal and uremic erythrocytes with high cell Na did not show any progressive changes in cell Na or K. In the presence of ouabain, the net gain of Na in normal and uremic erythrocytes was similar (1.21±0.19 vs. 1.08±0.20 mmol/liter cells per h).

Morphological and enzymatic characteristics of erythrocytes

The following indices of erythrocyte age were not different between normal subjects and patients with chronic renal failure: reticulocyte count, cell density, water content, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. G_6PD levels were higher in erythrocytes from dialysis patients as compared with normal subjects (9.44±1.50 vs. 7.73±1.57 U/g Hb, $P < 0.05$), but there was no difference in the level of this enzyme between dialysis patients with high cell Na and those with normal cell Na (9.72±1.73 vs. 8.87±0.73 U/g Hb). Thus, we found no evidence of an increase in mean cell age to explain reduced activity of Na-K pump in dialysis patients, and no difference in cell age between dialysis patients with high and normal cell Na. Less than 10% of erythrocytes from either subgroup of uremic subjects were abnormal on peripheral smear; spiculation, anisocytosis, and poikilocytosis were the most frequent abnormalities.

Quantitation of [^3H]ouabain-binding capacity on erythrocytes separated on Percoll density gradients

Fig. 4 shows the Na-K pump sites per cell plotted against erythrocyte G_6PD activity. In normal erythrocytes, the number of Na-K pump sites declined steeply with increasing cell age (declining G_6PD), so that the youngest normal erythrocytes from the top fractions possessed almost three times the number of Na-K pump sites per cell as compared with the older cells from the bottom fractions (open symbols, Fig. 4).

Erythrocytes from uremic subjects also showed an age-

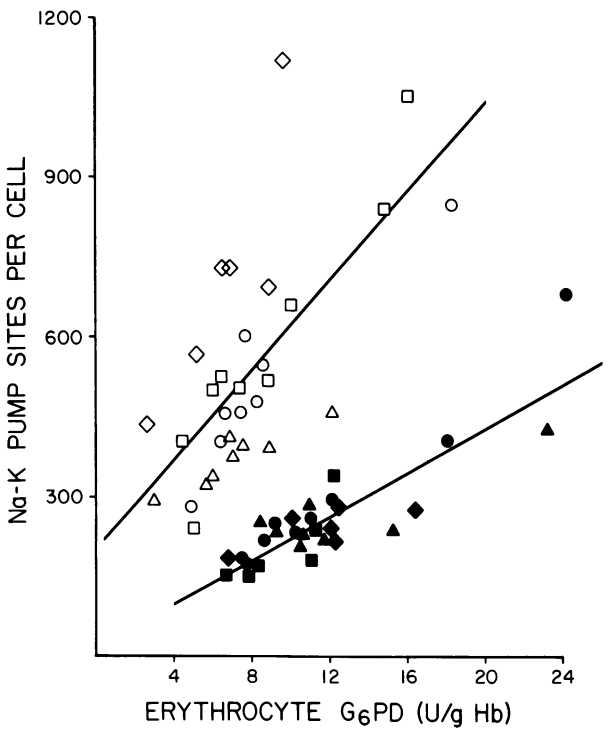

Figure 4. The number of Na-K pump sites, quantitated [^3H]ouabain binding, plotted against the erythrocyte G_6PD values. Erythrocytes were separated by density on continuous Percoll gradients. The open symbols denote normal subjects and the closed symbols denote uremic subjects. Each symbol denotes a different subject. The relationship for the normal group was $y = 42.0x + 203.9$, $r = 0.69$, and that for the uremic subjects was $y = 20.7x + 16.2$, $r = 0.87$. The two slopes were significantly different ($P < 0.05$). The correlation coefficient for each individual subject was greater than that for the entire group for both the normal and uremic subjects.

dependent decline in the number of Na-K pump sites (closed symbols, Fig. 4). For any given level of G_6PD, the [^{3}H]ouabain-binding capacity of uremic erythrocytes was lower than that of normal erythrocytes. The slope of the line correlating [^{3}H]ouabain-binding capacity and G_6PD was steeper in the normal as compared with the uremic group ($P < 0.01$) with a tendency towards divergence at higher levels of G_6PD (Fig. 3). Thus, [^{3}H]ouabain-binding capacity of younger uremic erythrocytes was appreciably less than that of younger normal erythrocytes.

Serial studies

Studies to show that high erythrocyte Na in group II B was an acquired abnormality. In 13 out of 16 patients from group II *B* (chronic renal failure-high cell sodium), intracellular Na was available before the onset of end-stage renal failure or shortly after the initiation of dialysis. In all 13 patients, intracellular Na was within the normal range at that time with a mean of 10.4±1.8 mmol/liter cell water. In two other patients with high cell Na who were reexamined 4–20 wk after the initial studies, a fall in cell Na to normal levels (9.7±1.15 vs. 16.2±5.0) was accompanied by an increase in [^{3}H]ouabain binding (416±60 vs. 320±13 sites/cell). Overall, 15 out of 16 patients in group II *B* (chronic renal failure-high cell Na) were found to have normal cell Na at one time, indicating that the elevated cell Na was an acquired defect and not secondary to genetic factors.

Studies to examine the effect of dialysis on the erythrocyte Na-K pump. Three patients not included above were found to have high intracellular Na before dialysis was ever initiated. In all three patients, intracellular Na was essentially unchanged after 3 wk of dialysis. After 6 wk of dialysis, intracellular Na decreased and [^{3}H]ouabain-binding capacity increased in all three patients. Fig. 5 depicts the time-course of improvement in intracellular Na and [^{3}H]ouabain binding in one of these patients.

In contrast to the long-term effects of dialysis, intracellular Na and [^{3}H]ouabain binding remained essentially unchanged after a single course of 4 h of hemodialysis in both the group with normal cell Na and the group with high cell Na (data not shown).

Clinical characteristics

Table II compares the clinical characteristics of dialysis patients with normal cell Na and dialysis patients with high cell Na. Group II *A* comprised of 24 blacks, 4 whites, and a hispanic, whereas group II *B* comprised of 15 blacks and one white.

Patients with high cell Na had higher serum creatinine (17.0±4.1 vs. 13.4±5.1 mg/dl, $P < 0.025$) and were on maintenance dialysis for a greater length of time (3.38±3.30 vs. 1.47±2.41 yr, $P < 0.05$). There was a significant direct correlation between serum creatinine (x) and erythrocyte Na concentration (y) ($y = 6.72 + 0.39x$, $r = 0.43$, $P < 0.01$). No correlation was detected between the duration of dialysis and cell Na. Hematocrit was 25.3±6.4% in the patients with high cell Na as compared with 21.3±3.4% in the group with normal

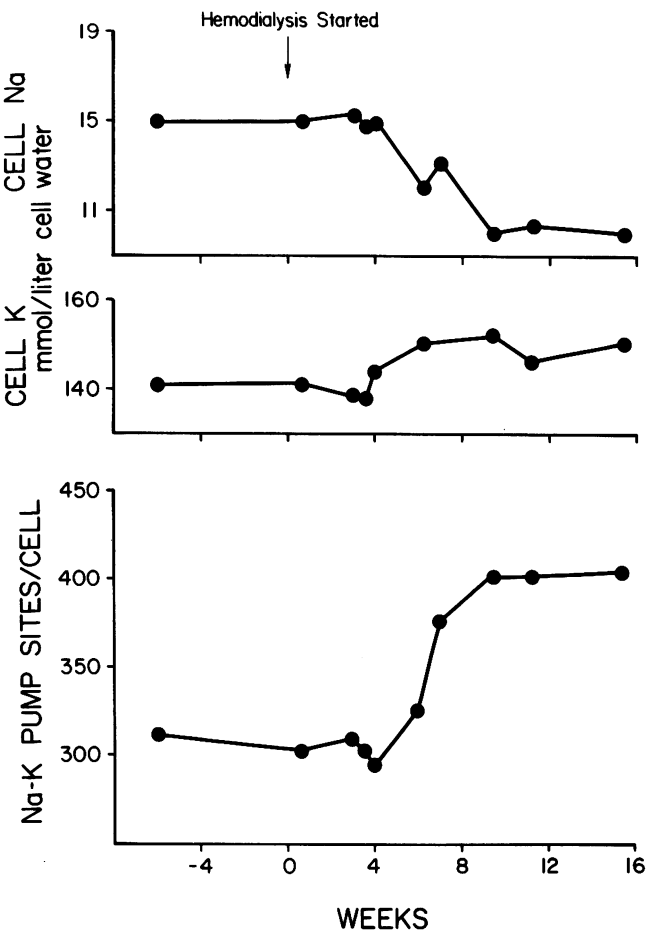

Figure 5. Serial measurement of intracellular Na (top), intracellular K (middle), and Na-K pump sites per cell (bottom) in a uremic subject before and after hemodialysis. Dialysis was started at time zero and continued throughout the course.

cell Na ($P < 0.05$). Patients with high cell Na were ambulant, free from intercurrent illness, and essentially asymptomatic. Five patients with high cell Na were judged to be inadequately dialyzed on clinical grounds. The other 11 patients with high cell Na and all patients with normal cell Na were judged to be adequately dialyzed. There was no difference in blood-urea nitrogen or blood pressure between the two groups.

Four patients from group II *A* and four patients from group II *B* had received one unit of packed cells each within 4 mo of the study. The shortest interval between the last transfusion and harvesting of blood for this study was 8 wk from group II *A* and 10 wk for group II *B*.

Patients in both subgroups were receiving aluminum hydroxide gels, multivitamins, ferrous sulfate, calcium carbonate, and vitamin D analogues. In addition, five patients from group II *A* and three patients from group II *B* were on antihypertensive medications including methyldopa, hydralazine, and propranolol.

Table II. Clinical and Biochemical Characteristics of Dialysis Patients with Normal and High Intracellular Na

	CRF patients with normal cell Na	CRF patients with high cell Na
Age (*yr*)	58.6±11.7	57.3±10.5
Duration on dialysis (*yr*)	1.47±2.41	3.38±3.30*
Height (*cm*)	173.6±7.1	169.9±10.7
Weight (*kg*)	68.03±12.9	60.1±16.9
Systolic blood pressure (*mmHg*)	145.6±23.4	140.7±24.8
Diastolic blood pressure (*mmHg*)	81.4±11.1	82.9±13.4
Dialysis prescription (m^2 *h/wk*)	15.03±2.60	13.94±1.94
Hematocrit (%)	21.3±3.4	25.3±6.4*
Blood urea nitrogen (*mg/dl*)	96.2±30.6	88.5±28.8
Serum creatinine (*mg/dl*)	13.4±5.1	17.0±4.1**
Total protein (*g/dl*)	6.9±0.7	7.0±0.7
Albumin (*g/dl*)	3.6±0.6	3.8±0.4
Serum Na (*mmol/liter*)	140.2±4.1	140.9±5.0
Serum Cl (*mmol/liter*)	103.9±5.1	100.6±5.8
Serum K (*mmol/liter*)	4.9±0.8	4.9±0.9
Serum HCO_3 (*mmol/liter*)	17.9±3.3	18.9±3.0
Serum Ca (*mg/dl*)	8.9±0.9	9.0±1.4
Serum P (*mg/dl*)	5.2±1.6	5.4±2.2
Alkaline phosphatase (*IU/liter*)	162.0±137.3	229.2±180.1
Parathyroid hormone, *N* terminal (*ng/ml*)	1.10±0.52	1.54±0.75
Parathyroid hormone *C* terminal (*ng/ml*)	7.11±4.04	7.06±4.39

* $P < 0.05$.
** $P < 0.025$
PTH measurements were made in 14 patients with normal cell Na and eight patients with high cell Na. All other parameters were measured in the entire subgroup of patients.

Discussion

The Na-K pump consists of a number of discrete ion pump units, which mediate the transport of Na and K across the cell membrane (1–4). A decrease in the number of Na-K pump units may lead to decreased Na efflux and a rise in intracellular Na, as illustrated by the inverse correlation between ion pump sites and cell Na in our normal subjects (Fig. 2). In uremic[2] subjects, the number of Na-K pump sites was significantly diminished in erythrocytes with high cell Na (Fig. 1). A significant inverse correlation between ion pump sites and intracellular Na was also detected in uremic subjects (Fig. 2). A change in the number of Na-K pump sites of erythrocytes from a given patient followed for several weeks was accompanied by a reciprocal change in intracellular Na (Fig. 5). Thus, in uremic subjects, as in normals, the number of Na-K pump sites appears to be an important determinant of intracellular Na.

A digoxin-like substance has been postulated to accumulate in the plasma of uremic patients (35). Such a substance could bind tightly or irreversibly to the membranes of uremic erythrocytes and interfere with the binding of [^{3}H]ouabain, thus leading to an underestimate of the number of pump sites. The presence of such a substance was investigated by measuring the change in the [^{3}H]ouabain-binding capacity of normal erythrocytes by exposure to uremic plasma. Uremic plasma failed to decrease the [^{3}H]ouabain-binding capacity of normal erythrocytes. Thus, there was no evidence for a digoxin-like substance in uremic plasma which could account for the decreased [^{3}H]ouabain-binding capacity of erythrocytes from patients with chronic renal failure.

In addition to the decrease in number of ion pump sites, a decrease in the ion turnover rate of individual Na-K pump sites may also lead to diminished Na efflux and an increase in intracellular Na concentration. Therefore, the ion turnover rates per pump site of uremic erythrocytes with high cell Na were measured and compared with the rates of normal erythrocytes. Comparison of ion turnover rates in intact cells is complicated by the fact that a change in intracellular Na itself influences the ion turnover rate of the Na-K pump site (20, 32–34). Thus, a valid comparison of ion turnover rates between normal erythrocytes and uremic cells with high cell Na requires that intracellular Na be equal in the two groups. Therefore, intracellular Na was altered and equalized at ~120 mmol/liter cell water in both groups with the help of nystatin. Under these conditions, the pump-mediated K influx was markedly lower in the uremic erythrocytes as compared with the normal erythrocytes (Table I, Fig. 3). The close correlation between the K pump influx and the number of Na-K pump sites (Fig. 3) indicates that the reduction of K transport across uremic cells was largely secondary to a decrease in the number of Na-K pump units. The ion turnover rate of individual Na-K pumps was similar in the two groups (Table I). This finding suggests that there is no intrinsic alteration in the transport capacity of individual Na-K pump units which could account for the rise in intracellular Na in uremic subjects.

When cation transport was evaluated in fresh cells without altering cell Na, K pump influx was similar in erythrocytes from normal subjects and from uremic subjects with high cell Na (Table I). Previous workers have shown that an increase in intracellular Na raises Na-K pump-mediated Na efflux and K influx in normal erythrocytes (20, 32–34). The finding of a similar, rather than raised K pump influx in uremic erythrocytes

2. The term "uremia," as used in this paper, denotes end-stage renal failure and does not imply adequacy or inadequacy of dialysis.

with high cell Na suggests that monovalent cation transport would be reduced in these cells if intracellular Na concentration was normal (5).

Based on the observations reported, our proposed scheme for the sequence of events leading to high cell Na is as follows. A reduced number of Na-K pump units per cell leads to reduced Na efflux initially. Because passive Na influx is normal, Na accumulates inside the cell. As cell Na rises progressively, the Na efflux is increased from subnormal to near normal levels, as a result of kinetic activation of the pump. The rise in cell Na continues until Na efflux matches the (largely) passive Na influx. In this new steady state, active Na efflux, active K influx, and passive permeability are all normal, but cell Na is raised. This is the state which was measured. The reduced number of pump units is the primary defect leading to reduced Na efflux and raised cell Na. The cells maintain raised Na levels because any reduction in the cell Na would be accompanied by a decrease in the active Na efflux and reaccumulation of cell Na. Because of the reduction in the pump units, normalcy of active cation fluxes can be maintained only at the expense of higher intracellular Na concentration.

It has been suggested that a factor in the uremic plasma may lead to a decrease in activity of the Na-K pump. In one previous study, Na-K-ATPase activity of normal erythrocytes exposed to uremic plasma was 16% lower than that of normal erythrocytes exposed to their own plasma (8). Others reported that Na efflux of normal erythrocytes was depressed by exposure to uremic plasma in at least one experiment in one patient (9). In the present studies, uremic plasma failed to depress the pump-mediated K transport of normal erythrocytes. Thus, we failed to confirm the presence of a putative uremic toxin which could directly depress the pump-mediated Na-K transport and raise intracellular Na in uremia.

A primary increase in passive Na influx across the cell membrane of uremic erythrocytes could also lead to an increase in cell Na, as suggested previously (12). In the present study, however, net Na influx was similar in normal and uremic erythrocytes in the presence of ouabain. The similarity of Na influx under conditions of complete inhibition of Na-K pump suggests that a difference in passive Na influx does not play a major role in raising intracellular Na of uremic erythrocytes.

The difference in correlation between the number of Na-K pump sites per cell and intracellular Na in normal and uremic subjects (Fig. 2) suggests that factors other than the decreased number of ion pump units may play an ancillary role in raising intracellular Na in uremia. We failed to demonstrate differences in the intrinsic ion turnover rates of individual Na-K pump units, a depression of cation transport by uremic plasma, or enhanced passive permeability for Na. Nonetheless, it is possible that other factors, such as an alteration in the affinity of the Na-K pump for substrates, may additionally impair Na transport and raise intracellular Na in uremia.

The decrease in [^{3}H]ouabain-binding capacity of uremic erythrocytes could either be due to a decrease in the synthesis of new Na-K pump sites or to an accelerated loss of Na-K pump sites during the life span of the erythrocytes in the peripheral circulation. Experiments on erythrocytes separated by age on Percoll gradients were designed to differentiate between these possibilities. The finding of a steep decline in the number of Na-K pump sites with increasing cell age in normal subjects (Fig. 4) confirms and extends earlier reports of higher [^{3}H]ouabain binding in reticulocyte-rich erythrocytes and younger cells reported previously (27, 36). The youngest uremic erythrocytes showed markedly lower [^{3}H]ouabain-binding capacity as compared with age-matched normal erythrocytes (Fig. 4), suggesting that the synthesis of Na-K pump sites is depressed in uremic subjects. If accelerated loss of Na-K pump units of erythrocytes during the aging process of the cell were responsible for the decrease in [^{3}H]ouabain-binding capacity of uremic cells, the slope of Na-K pump sites plotted against erythrocyte G_6PD would decline more steeply in the uremic cells. The failure to find a steeper slope in uremic erythrocytes argues against the hypothesis of accelerated Na-K pump loss in uremic erythrocytes during their life span. Instead, the slope was steeper in the normal subjects (Fig. 4). Since the fractional decay of Na-K pump sites with age was similar in the two groups, the greater decline in the number of Na-K pump sites in normal subjects with age was most likely consequent to the greater magnitude of Na-K pump units in the younger cells of normal subjects. Thus, a decrease in synthesis of Na-K pump sites of erythroid precursors may account for the decrease in [^{3}H]ouabain-binding capacity of erythrocytes from uremic subjects.

A fall in intracellular Na and a rise in the number of Na-K pump sites was observed in three patients after initiation of dialysis (Fig. 5). Earlier workers have reported an improvement in erythrocyte Na in one patient after several months of dialysis (8). Intracellular Na concentrations in the leukocyte and muscle cell have also been reported to fall after initiation of dialysis (10, 11). The lag period of several weeks between initiation of dialysis and the increase in the number of Na-K pump sites suggests that dialysis may permit increased synthesis of Na-K pump sites and thereby lead to an improvement in intracellular Na. The failure to notice an improvement in intracellular Na for 3 wk and the cotemporaneous improvement in cell Na and the number of Na-K pump sites favors the view that the decrease in the number of Na-K pump sites is the major factor responsible for the rise in cell Na in uremia.

Dialysis, however, is not always associated with an improvement in Na-K pump activity. 11 of our 16 patients in group II were judged to be adequately dialyzed, and yet, were found to have an elevation of intracellular Na. The persistence of the defect of Na-K pump on dialysis may reflect inadequate removal of the putative uremic toxin responsible for this defect. This possibility is supported by the finding of a significant direct correlation between serum creatinine and intracellular Na. In this context, it is noteworthy that a definition of

adequacy of dialysis is not readily available (37). The elevation of erythrocyte Na may itself be a sensitive index of the adequacy of dialysis (38).

It has been suggested that the variations in erythrocyte Na may be due to genetic differences (39–42). Genetic factors do not appear to be the etiology in our patients because normal intracellular Na was found at one time in 15 out of 16 patients with high cell Na. Since our patients with high cell Na were ambulatory, free from intercurrent and concurrent illness, and did not have evidence of an endocrine abnormality, the association of other diseases known to affect erythrocyte Na (6, 43) also did not play a role. Our finding that patients with high cell Na were on dialysis longer than those with normal cell Na (Table II) suggests that the abnormality of Na-K pump may increase over a period of time on maintenance dialysis.

In summary, we propose that a decrease in the synthesis of Na-K pump sites leads to a decrease in Na efflux and accumulation of intracellular Na inside the cells. The raised cell Na compensates for the decreased number of Na-K pump sites and restores the pump-mediated monovalent cation transport to normal levels. The decreased number of Na-K pump sites plays a major role in the abnormality of monovalent cation transport of erythrocytes from uremic subjects.

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