Altered Membrane Sodium Transport in Bartter's Syndrome

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A B S T R A C T   To explore the possibility that Bartter's syndrome is the manifestation of an inherited abnormality of sodium transport, we have measured various parameters of sodium transport in erythrocytes from patients with Bartter's syndrome, their siblings, and their parents. Sodium transport in six of the eight patients with Bartter's syndrome differed significantly from that in the other two patients. On the basis of this difference, the patients were divided into two groups (type I and type II). In the six type I patients, fractional sodium outflux (0.38±0.05/hr [SD]) was significantly less than normal (0.50±0.07) and erythrocyte sodium concentration (9.48±0.84 mmoles/liter cells per hr) was significantly greater than normal (5.24±0.66). In the two type II patients, none of the measured parameters of sodium transport differed significantly from normal. Erythrocyte sodium transport in the relatives of three type I patients was altered in a way similar to that in the type I patients and was significantly different from that in the relatives of a type II patient. These findings indicate the presence of inherited alterations of erythrocyte sodium transport in certain patients with Bartter's syndrome.

INTRODUCTION

In 1962 Bartter, Pronove, Gill, and MacCardle (1) described a syndrome characterized by idiopathic juxtaglomerular hyperplasia, hyperaldosteronism, hypokalemia, elevated concentrations of plasma renin and angiotensin, normal blood pressure, and the absence of peripheral edema. The etiology of this syndrome is unknown; however, vascular insensitivity to angiotensin (1), autonomous overproduction of renin (2), and defective sodium

reabsorption in the proximal renal tubule (3) have each been postulated to be the primary pathophysiologic event. Bartter's syndrome has been presumed to be a genetic disorder with an autosomal recessive pattern of inheritance (2) and has been reported to occur among siblings (4–6) and in one patient who was the product of a consanguineous union (6). To explore the possibility that Bartter's syndrome is a manifestation of a generalized, inherited abnormality of sodium transport, we have measured various parameters of sodium transport in erythrocytes from patients with Bartter's syndrome, their siblings and their parents.

METHODS

Fresh heparinized blood was obtained from 16 normal male and female human volunteers (ages 9–35), 8 patients with Bartter's syndrome (1), 8 parents, 8 siblings, 7 patients with secondary hyperaldosteronism from other causes (7, 8), and 1 patient with primary hyperaldosteronism (7) whose diagnosis was confirmed by subsequent surgical removal of an adrenal adenoma. Each of the patients with Bartter's syndrome demonstrated juxtaglomerular hyperplasia on renal biopsy, hypokalemia, elevated aldosterone secretory rate, increased plasma renin, normal blood pressure, the absence of peripheral edema, and a diminished vasopressor response to intravenous angiotensin infusion. Three of these patients with Bartter's syndrome have been the subjects of previous reports (1, 9, 10).

The cells were separated by centrifugation at 3000 g for 5 min at room temperature and the plasma and buffy coat removed by aspiration. Erythrocytes were then washed three times with iced isosmotic NaCl, divided into two portions, and placed in identical preincubation solutions (prewarmed to 37°C) at approximately 10% hematocrit. ²⁴Na was added to the preincubation mixture containing erythrocytes which were subsequently used for sodium outflux determinations. After 2 hr of incubation at 37°C in an oscillating water bath (American Instrument Co., Inc., Silver Springs, Md.), samples were taken for determination of hematocrit and hemoglobin concentration. The cells were then separated by centrifugation and washed three times with iced isosmotic choline chloride. A portion of the washed cells was hemolyzed and diluted for the determination of sodium and hemoglobin concentration. Hemoglobin was measured using the

cyanmethemoglobin method (11). Sodium concentration was measured with an Instrumentation Laboratory model 143 flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.). Erythrocyte sodium concentration was calculated after the method of Sachs and Welt (12).

The standard preincubation and incubation solutions had the following composition (mM): NaCl, 146; KCl, 6.0; tris buffer (pH = 7.4), 10; glucose, 11.1. Whenever the concentration of sodium or potassium was decreased, an equimolar amount of choline was substituted. Ethacrynic acid, when added to the incubation medium, was present at a final concentration of 1 mM.

Sodium outflux. The cells which were preincubated in the medium containing ^{24}Na were added to various incubation solutions (prewarmed to 37°C) at an hematocrit of approximately 5%. After mixing thoroughly, samples were taken at time "zero" and various intervals thereafter. The samples were chilled, the cells separated by centrifugation at 10,000 g for 15 sec, and a portion of the supernate was taken for measurement of radioactivity. At some time during the experiment, a portion of the incubation mixture (i.e., cells plus medium) was sampled. The cells were lysed and the protein was precipitated by adding 5% perchloric acid and centrifuging at 10,000 g for 30 sec. Appropriate control experiments demonstrated no significant entrapment of ^{24}Na in the precipitate. The supernate was added to a counting vial and its radioactivity measured. Initially samples were taken at 0, 15, 30, 45, and 60 min; however, since the fractional sodium outflux was found to be constant over this period, fractional sodium outflux was calculated from samples taken at 0 and 30 min. Radioactivity was determined with a Packard model 3320 liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

Fractional sodium outflux (k) was calculated from the following equation (13):

$$k = \frac{-\ln(A_{30}/A_0)}{t}$$

where k = fractional sodium outflux (hr^{-1}); A_0 and A_{30} = amount of radioactivity in a portion of cells at 0 and 30 min; t = times (hours).

Sodium influx. The cells which were preincubated in the nonradioactive medium were added to various prewarmed incubation solutions which contained ^{24}Na but were otherwise identical in composition to those used for outflux determination. The cells were mixed thoroughly and samples were taken at 0 and 30 min. The samples were chilled and the cells washed four times by alternate centrifugation and resuspension in iced isosmotic choline chloride. The cells were lysed and the protein precipitated by adding 5% perchloric acid and centrifuging at 10,000 g for 30 sec. The supernate was added to a counting vial and its radioactivity measured. At some time during the experiment, a portion of the incubation mixture was taken for determination of hemoglobin concentration. The volume of cells counted was calculated from the hemoglobin concentration of the incubation mixture and the previously measured hemoglobin content per volume of cells. Changes in cell water (determined gravimetrically) during the 30-min incubation period were negligible.

Sodium influx (J_{in}) was calculated from the following equation (13):

$$J_{in} = \left[\frac{kU}{1 - e^{-kt}}\right] \Big/ SA$$

where J_{in} = sodium influx (mmoles/liter cells per hour); U = uptake of radioactivity/liter of cells at time "t"; SA = specific activity of the incubation medium; t = time (hours).

Radioactivity was usually such that the standard deviation of the observed counts was 1% or less and all counts were corrected for decay. Liquid scintillation counting was performed using 20 ml of solution composed of 15 parts toluene (J. T. Baker Chemical Co., Phillipsburg, N. J.), 5 parts Triton X-100 (New England Nuclear Corp., Boston, Mass.) and 1 part Liquifluor (New England Nuclear Corp.).

To compare the specific activity of the sodium leaving the cells with the specific activity of the sodium in the cells, erythrocytes were incubated for 2 hr in the standard incubation medium containing ^{24}Na. The cells were then washed four times with cold, isosmotic choline chloride and incubated in a sodium-free medium for 1 hr. At 15-min intervals, samples were taken, chilled, and the sodium concentration and radioactivity were measured in the cells and in the supernate.

To study the effect of plasma from patients with Bartter's syndrome on erythrocyte sodium transport, fresh plasma and erythrocytes were obtained from three normal subjects and from three patients with Bartter's syndrome. The sodium and potassium concentrations of the plasma were adjusted to 146 and 6 mM, respectively. Erythrocytes from normal subjects and from patients with Bartter's syndrome were each preincubated for 3 hr with ^{24}Na in normal plasma and in Bartter's plasma. Each portion of cells was then washed four times and incubated for 30 min in the same plasma used for its preincubation. Erythrocyte sodium concentration and fractional sodium outflux were determined as described previously.

To measure erythrocyte lactate production, washed red cells were incubated in the standard incubation medium (hematocrit approximately 25%) and the appearance of lactate in the extracellular fluid was measured enzymatically[1] at 15-min intervals. Initial studies demonstrated that lactate production by erythrocytes from patients with Bartter's syndrome and from normal subjects was constant for at least 3 hr. Lactate production was calculated from samples taken after 30 and 90 min of incubation using the following equation:

$$P_L = \left[\frac{H}{1.0 - H}\right](L_{90} - L_{30})$$

where P_L = lactate production (mmoles/liter cells per hour); H = hematocrit of the incubation mixture; L_{30}, L_{90} = mmoles lactate/liter incubation mixture at 30, and 90 min respectively.

Unless otherwise specified, statistical comparisons were performed using Students' t test.

RESULTS

Table I summarizes the data for sodium transport in erythrocytes from patients with Bartter's syndrome, patients with secondary hyperaldosteronism from other causes, and one patient with primary hyperaldosteronism. Sodium transport in erythrocytes from six of the eight patients with Bartter's syndrome differed from that in the other two patients in terms of erythrocyte sodium concentration, fractional sodium outflux (in both the

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[1] Boehringer and Soehne Co., Mannheim, Germany. From Calbiochem, San Diego, Calif.

Subjects	Erythrocyte sodium concentration	Fractional sodium outflux			Sodium influx		
		Control*	+ETA‡	Change§	Control	+ETA	Change
	mmoles/ liter cells		hr⁻¹			mmoles/liter cells per hr	
Bartter's syndrome							
C. J. (19 M)	9.93	0.405	0.234	−0.171	1.69	1.95	0.26
K. B. (4 F)	10.99	0.343	0.208	−0.135	1.52	2.08	0.56
J. S. (2 F)	8.82	0.428	0.263	−0.165	1.74	1.98	0.24
M. J. (12 F)	8.86	0.300	0.162	−0.138	1.63	1.90	0.27
D. C. (13 F)	9.30	0.359	0.208	−0.151	1.64	2.11	0.47
J. E. (41 F)	8.99	0.432	0.253	−0.179	1.73	1.96	0.23
Mean±1sd	9.48±0.84‖	0.38±0.05‖	0.22±0.04‖	−0.16±0.03	1.66±0.08	2.00±0.08‖	0.34±0.14‖
J. A. (37 F)	5.56	0.537	0.365	−0.172	1.80	1.71	−0.09
R. B. (9 M)	5.16	0.526	0.313	−0.213	1.62	1.63	· 0.01
Mean±1sd	5.36±0.28	0.53±0.01	0.34±0.04	−0.19±0.03	1.71±0.13	1.67±0.06	−0.04±0.07
Normal subjects (16)	5.24±0.66	0.50±0.07	0.34±0.04	−0.16±0.03	1.74±0.20	1.70±0.28	−0.04±0.12
Secondary hyperaldo. (7)	5.52±0.73	0.51±0.05	0.33±0.02	−0.18±0.05	1.69±0.23	1.71±0.26	0.02±0.14
Primary hyperaldo. (1)	5.29	0.493	0.342	−0.151	1.68	1.71	0.03

The values for all subjects were determined in duplicate.
Age and sex of patients with Bartter's syndrome in parentheses.
* Control incubation medium (mM): Na, 146; K, 6.0.
‡ Ethacrynic acid (1 mM) added to control incubation medium.
§ Calculated by subtracting control value from that obtained when the medium contained 1 mM ethacrynic acid.
‖ Significantly different ($P < 0.01$) from mean value for patients J. A. and R. B. and from the mean value for normal subjects by t test.

presence and absence of ethacrynic acid), and the effect of ethacrynic acid on sodium influx. On the basis of these differences, the patients with Bartter's syndrome have been divided into two groups which we have termed type I and type II.

In the six type I patients, erythrocyte sodium concentration was significantly greater than normal and fractional sodium outflux, both in the presence and absence of ethacrynic acid, was significantly less than normal. Addition of ethacrynic acid to the incubation medium produced an equivalent decrease in fractional sodium outflux in type I patients compared with normal. Sodium influx in these six patients did not differ significantly from normal; however, addition of ethacrynic acid produced a significant increase in sodium influx in the type I patients but produced a negligible decrease in sodium influx in normal subjects. In the two type II patients, in the patients with secondary hyperaldosteronism from other causes and in the patient with primary hyperaldosteronism, none of the measured parameters of erythrocyte sodium transport differed significantly from normal.

When erythrocytes from four type I patients (C. J., K. B., M. J., and D. C.) were incubated in a sodium-free

medium, the average specific activity of the sodium leaving the cells (3608±65 cpm/nmole ±1 sd) was not significantly different from the average specific activity

TABLE II
Absence of an Effect of Plasma on Fractional Sodium Outflux

Subjects	Fractional Sodium outflux (hr⁻¹)	
	Normal plasma	Bartter's plasma
Bartter's syndrome		
C. J. (19 M)	0.396	0.382
K. B. (4 F)	0.340	0.346
D. C. (13 F)	0.352	0.351
Normals		
E. G. (20 M)	0.487	0.493
B. W. (18 F)	0.526	0.515
L. L. (9 F)	0.493	0.486

The values for all subjects were determined in duplicate.
Age and sex of subjects in parentheses.
The sodium and potassium concentrations of the plasma were adjusted to 146 and 6 mM respectively.
Erythrocytes from each subject were preincubated for 3 hr and incubated for 30 min in plasma from a normal subject and in plasma from a patient with Bartter's syndrome.

in the cells (3584±58). These data indicate that the source of the increased erythrocyte sodium concentration observed in these four patients was the decreased fractional sodium outflux. Garrahan and Glynn (13) have reported similar findings in erythrocytes from normal human subjects.

Table II illustrates that the decreased fractional sodium outflux in erythrocytes from three type I patients (C. J., K. B., and D. C.) persisted when the cells were preincubated and incubated in plasma from normal subjects. Similarly, plasma from these three patients did not alter fractional sodium outflux in erythrocytes from normal subjects.

Lactate production by erythrocytes from four type I patients (C. J., K. B., M. J., and J. E.) was 2.29±0.80 mmoles/liter cells per hr (mean ±1 SD) and was not significantly different from lactate production by erythrocytes from 10 normal subjects (1.92±0.59).

Table III summarizes the results of studies of sodium transport in erythrocytes from relatives of three type I patients and of one type II patient. Erythrocyte sodium transport in the relatives of type I patients was significantly different from that in the relatives of the type II patient in terms of fractional sodium outflux (in both the presence and absence of ethacrynic acid) and the effect of ethacrynic acid on sodium influx, but not in terms of erythrocyte sodium concentration. Sodium transport in the relatives of type I patients differed significantly ($P < 0.01$) from that in normal subjects in that erythrocyte sodium concentration was increased, fractional sodium outflux was decreased both in the presence and absence of ethacrynic acid and ethacrynic acid stimulated sodium influx. In the relatives of the type II patient, fractional sodium outflux in the presence of ethacrynic acid and sodium influx were significantly less than normal ($P < 0.01$, $P < 0.05$ respectively).

TABLE III

Sodium Transport in Erythrocytes from Relatives of Patients with Bartter's Syndrome

Subjects	Erythrocyte sodium concentration	Fractional sodium outflux			Sodium influx		
		Control*	+ETA‡	Change§	Control	+ETA	Change
	mmoles/ liter cells		*hr⁻¹*			*mmoles/liter cells per hr*	
C. J. family							
Father (50)	5.70	0.420	0.258	−0.162	1.70	2.04	0.34
Mother (45)	5.18	0.391	0.219	−0.172	1.55	1.86	0.31
R. J. (3 F)	7.29	0.346	0.156	−0.190	1.56	1.58	0.02
D. J. (14 F)	6.42	0.350	0.211	−0.139	1.58	1.89	0.31
T. J. (9 M)	6.47	0.347	0.197	−0.170	1.58	1.65	0.07
R. J. (16 M)	7.38	0.372	0.219	−0.153	1.57	1.83	0.26
K. B. family							
Father (23)	7.19	0.372	0.246	−0.126	1.94	2.04	0.10
Mother (23)	9.00	0.272	0.156	−0.116	1.69	1.77	0.08
T. B. (2 M)	8.68	0.318	0.200	−0.118	1.59	2.05	0.46
J. S. family							
Father (38)	7.38	0.430	0.276	−0.154	1.98	2.11	0.13
Mother (26)	5.28	0.419	0.303	−0.116	1.67	1.70	0.03
Mean±1 SD	6.91±1.25	0.37±0.05	0.22±0.05	−0.15±0.02	1.67±0.15	1.86±0.18	0.19±0.15
R. B. family							
Father (42)	6.60	0.487	0.268	−0.219	1.86	1.61	−0.25
Mother (34)	5.52	0.468	0.271	−0.197	1.20	1.28	0.08
S. B. (5 M)	5.37	0.447	0.308	−0.139	1.40	1.44	0.04
J. B. (6 F)	5.89	0.468	0.286	−0.182	1.66	1.58	−0.08
M. B. (11 F)	5.38	0.448	0.268	−0.180	1.39	1.34	−0.05
Mean±1 SD	5.75±0.52	0.46±0.02‖	0.28±0.02‖	−0.18±0.03	1.50±0.26	1.45±0.14‖	−0.05±0.13‖

The values for all subjects were determined in duplicate.
Age and sex of subjects in parentheses.
* Control incubation medium (mM): Na, 146; K, 6.0.
‡ Ethacrynic acid (1 mM) added to control incubation medium.
§ Calculated by subtracting control value from that obtained when the medium contained 1 mM ethacrynic acid.
‖ Significantly different ($P < 0.02$) from mean value for all type I relatives.

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TABLE IV
Sodium Transport in Erythrocytes from Parents and Siblings of Patients with Bartter's Syndrome

	Erythrocyte sodium concentration	Fractional sodium outflux			Sodium influx		
		Control*	+ETA‡	Change§	Control	+ETA	Change
	mmoles/ liter cells	hr⁻¹			mmoles/liter cells per hr		
Siblings							
Type I (5)	7.25±0.92‖	0.35±0.02‖	0.20±0.02‖	−0.15±0.03	1.58±0.01	1.80±0.19‖	0.22±0.18
Type II (3)	5.55±0.30	0.45±0.01	0.29±0.02	−0.17±0.02	1.48±0.15	1.45±0.12	−0.03±0.06
Parents							
Type I (6)	6.62±1.50	0.38±0.06	0.24±0.05	−0.14±0.02¶	1.76±0.17	1.92±0.17‖	0.16±0.12
Type II (2)	6.06±0.76	0.48±0.01	0.27±0.002	−0.21±0.02	1.53±0.47	1.44±0.23	−0.08±0.23

All values expressed as mean ±1 SD.
* Control incubation medium (mM): Na, 146; K, 6.0.
‡ Ethacrynic acid (1 mM) added to control incubation medium.
§ Calculated by subtracting control value from that obtained when the medium contained 1 mM ethacrynic acid.
‖ Significantly different ($P < 0.02$) from mean value for siblings of patient R. B.
¶ Significantly different ($P < 0.02$) from mean value for parents of patient R. B.

When each group of relatives was subdivided into siblings and parents (Table IV), sodium transport in erythrocytes from siblings of type I patients was significantly different from that in the siblings of the type II patient in terms of erythrocyte sodium concentration, fractional sodium outflux and sodium influx in the presence of ethacrynic acid. Sodium transport in erythrocytes from siblings of type I patients differed significantly ($P < 0.01$) from that in normal subjects in that erythrocyte sodium concentration was increased, fractional sodium outflux was decreased both in the presence and absence of ethacrynic acid, and ethacrynic acid stimulated sodium influx. In siblings of the type II patient, fractional sodium outflux in the presence of ethacrynic acid and sodium influx were decreased ($P < 0.05$) compared with normal subjects.

Sodium transport in erythrocytes from parents of type I patients was significantly different from that in the parents of the type II patient in terms of the decrease in fractional sodium outflux produced by adding ethacrynic acid to the medium and in terms of sodium influx in the presence of ethacrynic acid. Although these two groups did not differ significantly ($P < 0.05$) with respect to the mean control fractional outflux values, all of the values for the parents of the type I patient were lower than the values for the parents of the type II patient. Sodium transport in erythrocytes from parents of the type I patients differed significantly ($P < 0.01$) from that in normal subjects in that erythrocyte sodium concentration was increased, fractional sodium outflux was decreased both in the presence and absence of ethacrynic acid, and ethacrynic acid stimulated sodium influx. In the parents of the type II patient fractional sodium outflux in the presence of ethacrynic acid was below normal ($P < 0.05$) and the decrease in fractional sodium outflux produced by adding ethacrynic acid to the medium was greater than normal ($P < 0.05$).

DISCUSSION

The altered erythrocyte sodium transport observed in the six type I patients cannot be attributed to hypokalemia or to elevated plasma concentrations of renin, angiotensin, or aldosterone since erythrocyte sodium transport was normal in the two type II patients with Bartter's syndrome and in the seven patients with secondary hyperaldosteronism from other causes. This conclusion is given additional support by previous studies from other laboratories (4, 14, 15). The observations that plasma from type I patients had no significant effect on fractional sodium outflux in normal erythrocytes and that the altered sodium transport in erythrocytes from type I patients persisted in plasma from normal subjects indicate that the altered erythrocyte sodium transport in type I patients represents an intrinsic abnormality of their erythrocytes. We should mention, however, that these data do not exclude the rather unlikely existence in the type I patients of a circulating factor which interacts so slowly with erythrocytes that its effect can be neither reduced in erythrocytes from type I patients nor exerted on normal erythrocytes during the 3 hr incubation period. The normal values for lactate production by erythrocytes from four of the type I patients exclude a defect in glycolysis as the source of the altered sodium transport. We are unable to explain the observation that ethacrynic acid increased sodium influx in the type I patients; however, this effect constitutes a qualitative difference whose understanding may

help clarify the nature of the basic abnormality in these patients.

We are unable to reconcile the discrepancy between our findings and those of Gall, Vaitukaitis, Haddow, and Klein (16, 17) who reported a patient with Bartter's syndrome in whom erythrocyte sodium concentration, fractional sodium outflux, and sodium influx were increased. One possibility is that their patient (in whom juxtaglomerular hyperplasia was not demonstrated) had a syndrome whose basic etiology differed from that of the subjects used for our investigations. It is also possible that the discrepancy results from methodologic differences; however, the techniques used by Gall and coworkers are not described in sufficient detail to allow us to make a more precise statement. Finally, these authors' data for sodium transport in erythrocytes from a patient with Bartter's syndrome appear to be inconsistent. The 50% increase in sodium influx (which they suggest is the basic abnormality) is not of sufficient magnitude to account for an elevated erythrocyte sodium concentration when there is also an 80% increase in fractional sodium outflux. We were also unable to confirm these authors' observation of elevated fractional sodium outflux in erythrocytes from patients with primary and secondary hyperaldosteronism.

Others (9) have pointed out that with the exception of the cardinal features shared by all cases (juxtaglomerular hyperplasia, secondary hyperaldosteronism, hypokalemia, alkalosis, normal blood pressure, and absence of edema) there is marked variation in the physiologic and morphologic abnormalities in Bartter's syndrome. Our eight patients with Bartter's syndrome could be classified on the basis of their values for erythrocyte sodium transport: type I patients had increased erythrocyte sodium concentration and decreased fractional sodium outflux; type II patients had normal erythrocyte sodium concentration and fractional sodium outflux. We could find no correlation between the presence or absence of altered erythrocyte sodium transport and any of the other previously described physiologic or morphologic abnormalities in this clinical disorder (1, 3, 6, 9). Furthermore, the significance of classifying patients with Bartter's syndrome on the basis of their values for erythrocyte sodium transport must await clarification of the relation, if any, between altered erythrocyte sodium transport and the primary defect in this disorder.

Our finding of a similar alteration of sodium transport in six patients with Bartter's syndrome and in their relatives suggests that the defect in erythrocyte sodium transport is inherited. Although erythrocyte sodium concentration and fractional sodium outflux in R. B. and his relatives were normal, the patient's older sister (M. B.) has been previously demonstrated to have a diminished vasopressor response to angiotensin,

an abnormally high aldosterone excretion while on a low sodium diet and a slight decrease in her serum potassium level (10). If the alterations of erythrocyte sodium transport are related to the etiologic abnormality in Bartter's syndrome, this disorder may be analogous to cystinuria where patients, all of whom have increased urinary cystine, lysine, and arginine, can be divided into three types depending on their values for the intestinal uptake of lysine, arginine, and cystine and values for urinary cystine excretion in their parents (18).

Our data are not sufficient to allow us to specify the mode of inheritance of the altered erythrocyte sodium transport in the type I patients and their relatives. We are also unable to relate the observed alterations in erythrocyte sodium transport to the primary defect in Bartter's syndrome; however, our data do suggest that the clinical features of this syndrome may be manifestations of an inherited defect in membrane cation transport.

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