

Increased Erythrocyte Cation Permeability in Thalassemia and Conditions of Marrow Stress

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Research Article

Calcium and sodium permeability of erythrocytes from patients with untransfused α - or β - thalassemia major has been studied and compared to mature erythrocytes or control cells with comparable reticulocytosis. Isotopic Na^+ influx was increased a mean fourfold greater than normals and threefold greater than reticulocyte rich control. Passive net leak of Na^+ into thalassemic cells incubated with ouabain was also increased corresponding to their greater $^{22}\text{Na}^+$ influx. Erythrocyte Na^+ and K^+ concentrations and cell water content per unit volume of cells were normal. Quantitation of active cation pumps in the cell membrane by the technique of [^3H]ouabain binding showed a 2.6- to 9.9-fold increase above normal. Inward Ca^{2+} movement was studied in cells with absent Ca^{2+} pumping produced by depletion of either ATP or Mg^{2+} -ions. Calcium uptake by ATP depleted thalassemic cells was increased 12-fold above normals and 3.6-fold above reticulocyte-rich controls. The Ca^{2+} uptake by Mg^{2+} -depleted thalassemic cells was also increased above normal confirming that erythrocyte Ca^{2+} permeability is increased in this disease. Osmotic fragility measurements show that the surface area to volume ratio of thalassemic erythrocytes was increased by 15 to 25% above mature erythrocytes. The increased passive cation permeability of thalassemic erythrocytes cannot be explained by either reticulocytosis or an increased surface area to volume ratio of these cells. Moreover, erythrocyte Na^+ and Ca^{2+} influxes in congenital [...]

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ABSTRACT Calcium and sodium permeability of erythrocytes from patients with untransfused α - or β -thalassemia major has been studied and compared to mature erythrocytes or control cells with comparable reticulocytosis. Isotopic Na^+ influx was increased a mean fourfold greater than normals and threefold greater than reticulocyte rich control. Passive net leak of Na^+ into thalassemic cells incubated with ouabain was also increased corresponding to their greater $^{22}\text{Na}^+$ influx. Erythrocyte Na^+ and K^+ concentrations and cell water content per unit volume of cells were normal. Quantitation of active cation pumps in the cell membrane by the technique of [^3H]ouabain binding showed a 2.6- to 9.9-fold increase above normal. Inward Ca^{2+} movement was studied in cells with absent Ca^{2+} pumping produced by depletion of either ATP or Mg^{2+} -ions. Calcium uptake by ATP depleted thalassemic cells was increased 12-fold above normals and 3.6-fold above reticulocyte-rich controls. The Ca^{2+} uptake by Mg^{2+} -depleted thalassemic cells was also increased above normal confirming that erythrocyte Ca^{2+} permeability is increased in this disease. Osmotic fragility measurements show that the surface area to volume ratio of thalassemic erythrocytes was increased by 15 to 25% above mature erythrocytes. The increased passive cation permeability of thalassemic erythrocytes cannot be explained by either reticulocytosis or an increased surface area to volume ratio of these cells. Moreover, erythrocyte Na^+ and Ca^{2+} influxes in congenital dyserythropoietic anemia (CDA type 2) were increased 2- and 14-fold, respectively, above normal. The increased cation fluxes and cation pump numbers in thalassemic and congenital dyserythropoietic anemia erythrocytes are consistent with the hypothesis of membrane immaturity arising from rapid marrow transit times, a concept previously advanced to explain the persistence of i-antigen on these cells.

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INTRODUCTION

Thalassemia is a heterogeneous group of inherited diseases in which erythroid cells fail to synthesize the globin chains of hemoglobin. A number of erythrocyte membrane abnormalities have been documented in the thalassemic syndromes. First, the osmotic fragility is reduced so that the cells have an increased surface area to volume ratio. (1, 2). Second, the amount of i-antigen is increased on the circulating erythrocytes and the persistence of this fetal antigen has led to the suggestion that the erythrocyte membrane is immature (3, 4). Finally permeability studies have established that K^+ fluxes are increased both into and out of the erythrocyte although the intracellular K^+ concentrations are unknown (5). In this study the Na^+ and Ca^{2+} permeability as well as the binding of [^3H]ouabain to erythrocytes has been measured in patients with both α - and β -thalassemia major.

METHODS

Patients. F.L. was a black female with β -thalassemia major who had been splenectomized at the age of 8 yr with a decrease in her transfusion requirements. Blood for this study was always drawn 10 wk after preceding transfusions when her only medication was folic acid. An increased titer of i-antigen was present on her erythrocytes and numerous erythroblasts were present in the peripheral blood. (Table I). Four other patients with β -thalassemia major have been described previously, all had intact spleens, and none were transfusion dependent (6, 7). A.B. was an Italian girl diagnosed as β -thalassemia major in a family study after an older sibling was found to have the same disease. Hepatosplenomegaly was present and both parents were shown to have β -thalassemia minor. M.L. was a Chinese female student with hemoglobin H disease and splenomegaly who was asymptomatic at the time of study. C.C. was a Greek Cypriot woman with asymptomatic hemoglobin H disease and splenomegaly. An increased i-antigen was found on her erythrocytes. G.K. was a consanguineous white male with congenital dyserythropoietic anemia (type 2) with splenomegaly who was not transfusion dependent (Table I). His erythrocytes carried increased amount of i-antigen and 30% of the marrow erythroblasts were binucleate (8). The two patients with hereditary persistence

TABLE I
Hematological Parameters in Patients Studied

			Hemoglobin	Retics	MCV	Percent hemoglobin			Reference
						A ₂	F	H	
			g/dl	%	f				
F.L.*	19	Black	7.3	4.9	74	6.0	30	—	—
K.B.	18	White	8.0	5.6	60	3.9	11.8	—	6
C.B.	12	White	6.9	6.0	56	4.8	6.2	—	6
H.H.	13	Black	10.0	2.5	58	7.5	45.7	—	7
D.H.	10	Black	7.8	3.2	58	6.5	43.5	—	7
A.B.	7	White	9.7	3.5	71	6.0	63	—	—
M.L.	26	Chinese	9.8	2.5	60	—	—	8	—
C.C.	50	White	8.8	3.1	63	—	—	11	—
G.K.	41	White	9.5	4.1	113	2.0	1.9	—	8

* Patient splenectomized and washed erythrocyte suspensions contained 2–4% erythroblasts. This patient was studied 10 wk after preceding transfusion. No other patients were receiving transfusions.

of i-antigen were both hematologically normal although both possessed anti-I antibody. The control suspensions with 4.2% reticulocytes were obtained from a patient with autoimmune hemolytic anemia on no medication at the time of study.

Serology. The strength of i-antigen was quantitated by scoring erythrocyte agglutination in tubes containing doubling dilutions of two different anti-i antisera and comparing with pooled adult and pooled cord erythrocytes (9).

Permeability studies. Venous blood was drawn into heparin anticoagulant and erythrocytes separated from buffy coat by thrice washing at 4°C in a saline medium of composition of 145 mM NaCl, 5 mM KCl, and 20 mM imidazole Cl, pH 7.5. The reticulocyte counts shown in Table I as well as osmotic fragility measurements were performed on the washed erythrocytes by standard techniques (2). Erythrocyte Na⁺, K⁺, and water content, as well as isotopic sodium influx, and [³H]ouabain binding to erythrocytes were measured as described previously (10, 11). The net Na⁺ uptake of cells was measured over a 6-h incubation at 37°C with 5 mM glucose and 0.1 mM ouabain. Aliquots of the cell suspension were taken at 0, 2, 4, and 6 h, the cells rapidly washed fourfold in ice-cold 110 mM-MgCl₂, the cells were hemolyzed in 0.01 N-NH₄OH and Na⁺ analyzed by flame photometry (11).

Calcium uptake. Washed erythrocytes were depleted of ATP by preincubation for 90 min in medium plus 1 mM iodoacetate and 10 mM inosine (10, 12). The erythrocytes were then washed twice and added to prewarmed media of composition 145 mM NaCl, 5 mM KCl, 20 mM imidazole Cl, pH 7.5 plus 1.5 mM ⁴⁵CaCl₂ (1 μCi/ml). Samples were taken after 15 min and after 1, 2, 3, and 4 h, and washed four times in cold 150 mM NaCl. Each cell pellet was hemolyzed in 0.01 N NH₄OH, and part of the hemolysate was deproteinized with 6% (wt/vol) perchloric acid and ⁴⁵Ca²⁺ in the supernate was measured by liquid scintillation counting. To convert the uptake of radioactive calcium from nanomoles per micromole Hb into nanomoles per milliliter cells, the mean corpuscular hemoglobin concentration was measured in each experiment both for abnormal and normal cells.

Washed erythrocytes were depleted of Mg²⁺ by a 37°C preincubation in the dark for 2 h at 2% hematocrit in the above washing medium plus 10 mM glucose, 2 mM EDTA, and 17.5 nmol/ml cells of ionophore A23187 (Eli Lilly & Co., Indianapolis, Ind. (13). The erythrocytes were then washed five times at 37°C in saline medium plus 2 mM EDTA, 10 mM glucose,

and 4% bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, Mo.). They were then washed four more times at room temperature in a medium comprising 145 mM NaCl, 5 mM KCl, and 20 mM imidazole, pH 7.4. The internal Mg²⁺ concentration was determined by atomic absorption spectrophotometry and was within the range of 0.02 to 0.05 μmol Mg²⁺/ml cells. ⁴⁵Ca²⁺ uptake was measured on the washed, Mg²⁺-depleted erythrocytes as described above except for the addition of 5 mM inosine to the ⁴⁵Ca²⁺ containing medium.

Statistics. Mean values ± 1 SD are shown and differences between sample means analyzed by a *t* test.

RESULTS

Isotopic and net sodium influx. Unidirectional ²²Na⁺ influx into erythrocytes from six patients with β-thalassemia major was increased three- to eightfold above the normal value. Five of these influx values fell within the narrow range of 5.4 to 8.4 μeq/ml cells per h, whereas the highest value of 17.0 μeq/ml cells per h was for the patient with 4% circulating erythroblasts following a splenectomy. Two patients with hemoglobin H disease (α-thalassemia) showed an approximate twofold increase in erythrocyte ²²Na⁺ influx (Table II). The mean influx for all thalassemics, even excluding the patient postsplenectomy was significantly greater than the normal mean of 2.0 ± 0.2 μeq/ml cells per h (*P* < 0.001). The inhibition of ²²Na⁺ influx by the diuretic furosemide (1 mM) was 0.3 to 0.6 μeq/ml cells per h, a decrement that is not different to that observed in normal erythrocytes. Control erythrocytes, with comparable reticulocytosis (2.5 to 6.0%) had a total ²²Na⁺ influx between 2.1 and 2.8 μeq/ml cells per h.

Isotopic ²²Na entry into cells occurs not only by net downhill movement of this cation ("passive leak") but also by a carrier which mediates Na-Na exchange diffusion (14). The passive Na⁺ leak was measured by the net gain of Na⁺ by cells incubated with ouabain

TABLE II
Cation Concentrations, Sodium Influx and Ouabain Binding in Erythrocytes

	Erythrocytes		Na ⁺ influx <i>μeq/ml cells per h</i>	Maximum ouabain binding sites <i>molecules/cell</i>
	Na ⁺ <i>μeq/ml cells</i>	K ⁺ <i>μeq/ml cells</i>		
β-Thalassemia				
F.L.	13.1	103	17.0	1,275
K.B.	6.2	107	5.8	2,098
C.B.	—	—	8.4	3,594
H.H.	9.8	103	7.1	961
D.H.	6.7	102	6.2	—
A.B.	4.7	93	5.4	—
α-Thalassemia				
M.L.	6.7	111	4.2	—
C.C.	9.0	110	3.5	—
Mean for all thalassemia	8.0	104	7.2*	1,982*
Congenital dyserythropoietic anemia				
G.K.	9.4	102	4.0	—
Reticulocyte-rich (4.2%) control				
J.B.	—	—	2.8	811
Normals	7.9	99	2.0	362
SD	±1.5	±5	±0.2	±69
n	27	27	24	7

* Significantly increased above normal mean, $P < 0.001$.

to inhibit active cation transport. Erythrocytes from two patients with either α - or β -thalassemia (C.C. and A.B.) gained Na⁺ during a 6-h incubation at mean rates of 2.25 and 1.7 $\mu\text{eq/ml cells per h}$, respectively, compared with 0.85 $\mu\text{eq/ml cells per h}$ for normals (Fig. 1). Control erythrocytes from a patient with reticulocytosis of 4.7% following acute blood loss were incubated for 6 h with ouabain and gained Na⁺ at a mean rate of 0.95 $\mu\text{eq/ml cells per h}$.

Intracellular cation and water contents. Thalassemic erythrocytes had a normal concentration of Na⁺ and K⁺ expressed per unit volume of erythrocytes and although there was a slight increase in erythrocyte Na⁺ in the patient with circulating erythroblasts the mean cell Na⁺ for the entire group did not differ significantly from normal (Table II). Erythrocyte water content was also normal. One patient with β -thalassemia had 654 mg/g, two patients with α -thalassemia 652 and 665 mg/g, respectively, while normal water content was 658 ± 6 mg/g wet wt cells (11).

[³H]ouabain binding. Thalassemic erythrocytes bound more [³H]ouabain than normal erythrocytes, by a factor of 2.6- to 9.9-fold (Table II). This increase in ouabain binding was even greater if values were ex-

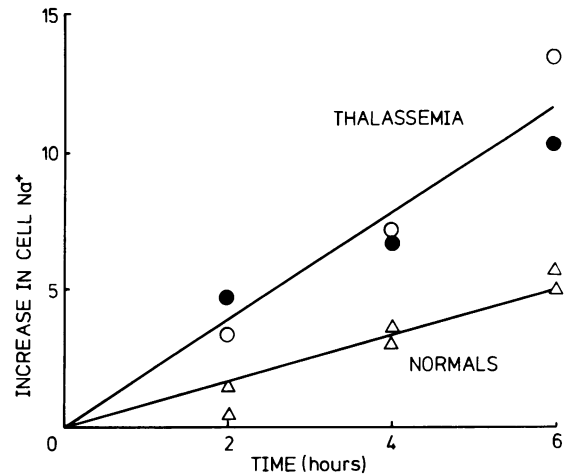


FIGURE 1 Net Na⁺ uptake by thalassemic erythrocytes in the presence of ouabain. Fresh cells were incubated at 37°C in a saline medium containing glucose plus 0.1 mM ouabain. The initial cell Na⁺ concentration of 5.9 to 8.8 $\mu\text{eq/ml cells}$ remained constant in a parallel incubation without ouabain. β -thalassemia (●) and α -thalassemia (○) suspensions contained 3.0 and 2.9% reticulocytes, respectively. The net Na⁺ uptakes for two normal subjects (△) occurs at a rate similar to published values (Fig. 4, 15).

pressed in sites per ml cells rather than sites per cell. The relationship between maximum [³H]ouabain binding and reticulocyte numbers suggests a value of 500–800 sites per cell in suspensions with 3–6% reticulocytes (10). Thus ouabain-binding sites on thalassemic cells were greater than expected for the percent reticulocytes present.

Calcium influx. Thalassemic erythrocytes showed a large increase in passive Ca²⁺ uptake, measured in cells depleted of ATP to inhibit the outward pumping of Ca²⁺ ions (12). The increase in Ca²⁺ uptake ranged from 6- to 15-fold (mean 12-fold) above the values for normal erythrocytes, and was observed with α - as well as β -thalassemic cells (Fig. 2). In one patient with α -thalassemia (C.C.) the erythrocytes were depleted of ATP in paired flasks by two different combinations of inhibitors, namely iodoacetate plus inosine or iodoacetamide plus inosine. Subsequent uptake of ⁴⁵Ca by cells depleted in these two ways was identical and both were elevated sevenfold above normal. (Isotopic Na⁺ influx in either the ATP-depleted cells or the Mg²⁺-depleted cells were never increased above values for ATP-rich cells.) Reticulocytes also have a Ca²⁺ permeability that is greater than that of mature erythrocytes (10). However, Ca²⁺ uptake into control cells with 4.2% reticulocytosis was only 3.7-fold above normal values (Fig. 2). Thus the large Ca²⁺ uptake by thalassemic erythrocytes is only partly explained by the mild reticulocytosis.

The passive uptake of Ca²⁺ was also measured in cells depleted of Mg²⁺ by ionophore (13) a procedure which

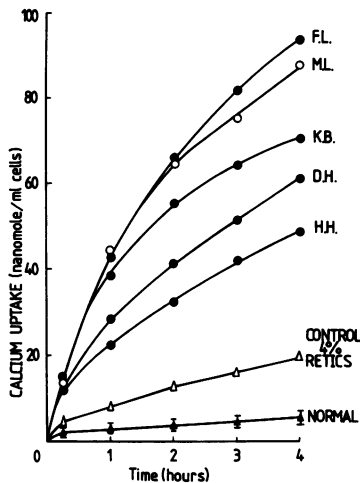


FIGURE 2 Calcium uptake by thalassemic erythrocytes. Cells were depleted of ATP and incubated at 37°C in a saline medium containing 1.5 mM $^{45}\text{CaCl}_2$. β -thalassemia (●) and α -thalassemia (○) suspensions contained 2.5–6.0% reticulocytes, while the control (△) from a patient with autoimmune hemolytic anemia contained 4.2% reticulocytes. Values for normal erythrocytes (▲) were from 14 normal donors and bars show ± 1 SD.

inhibits the outward extrusion of Ca^{2+} ions by removal of a different cofactor for the pump. The Ca^{2+} uptake of Mg^{2+} -depleted cells was $\sim 70\%$ the value for ATP-depleted cells in all experiments with thalassemic and normal subjects. The marked difference between normal and thalassemic erythrocytes was still observed since Ca^{2+} uptake by the Mg^{2+} -depleted cells from patient C.C. was increased by a factor of sixfold over normal (Table III). No increase in isotopic Na^+ influx into Mg^{2+} -depleted cells was found so that the depletion procedure did not perturb the passive cation permeability.

Osmotic fragility in thalassemia. Mean osmotic fragility for three patients with thalassemia were 0.31, 0.33, and 0.34 g NaCl/dl compared with a normal range of 0.40 to 0.44 g NaCl/dl. Thus thalassemic erythrocytes have a 15–25% increase in their surface area to volume ratio.

Congenital dyserythropoietic anemia (CDA).¹ Erythrocytes from a patient with CDA Type 2 also demonstrated an increased Na^+ and Ca^{2+} permeability that was greater than expected for the 4.1% reticulocytes present. Sodium influx was twofold normal (Table II), the intracellular Na^+ and K^+ concentrations were both normal and Ca^{2+} uptake was increased to the same extent as for thalassemic cells and reached 78 nmol/ml cells by 4 h, which is fourfold greater than the reticulocyte-rich control.

¹Abbreviations used in this paper: CDA, congenital dyserythropoietic anemia.

TABLE III
Calcium Uptake by Erythrocytes Depleted
Either of ATP or Mg^{2+} -Ions

	Ca^{2+} Uptake by ATP depleted cells			Ca^{2+} Uptake by Mg^{2+} depleted cells*		
	1 h	2 h	3 h	1 h	2 h	3 h
	nmol/ml cells			nmol/ml cells		
Normal (J.W.)	2.1	3.4	4.3	1.9	2.6	2.9
α -Thalassemia (C.C.)	16.1	23.7	26.7	10.0	12.2	18.7
Relative increase in Ca^{2+} uptake in thalassemia	7.7	7.0	6.2	5.3	4.7	6.4

* Normal and thalassemic erythrocytes were preincubated with the ionophore A23187 to deplete Mg^{2+} from 1.96–1.98 $\mu\text{mol/ml}$ cells down to 0.034 and 0.052 $\mu\text{mol/ml}$ cells, respectively. Reticulocyte count of the thalassemic cells was 1.2% after the washing prior to depletion.

Hereditary persistence of *i*-antigen. Cation fluxes were also measured in erythrocytes from two subjects with the rare anomaly of hereditary persistence of *i*-antigen (16). Sodium influx (1.9 and 2.0 $\mu\text{eq/ml}$ cells per h), and erythrocyte Na^+ and K^+ concentrations of 8.8 and 103 $\mu\text{eq/ml}$ cells, respectively, were all normal. Moreover Ca^{2+} uptakes by ATP-depleted cells from the two subjects (5.2 and 6.8 nmol/ml cells per 4 h) were also within the range for normal mature erythrocytes (5.4 ± 1.5 nmol/ml cells per 4 h).

DISCUSSION

The major finding is that thalassemic erythrocytes have a 4- and 12-fold increase in passive permeability to Na^+ and Ca^{2+} , respectively, as well as a similar increase in the number of sodium pumps per cell. Despite the substantial increase in Na^+ influx, intracellular Na^+ and K^+ concentrations were normal when expressed either per unit volume of cells or per unit of cell water. Clearly the increment in cation pumps was sufficient to compensate fully for the greater Na^+ influx. Erythrocyte K^+ fluxes, both influx and efflux are also known to be increased in thalassemia but although passive K^+ loss may be as much as eightfold normal, active K^+ influx is sufficient to maintain the initial K^+ concentration (5). In mature erythrocytes passive Na^+ and K^+ fluxes are partly mediated by a ($\text{Na} + \text{K}$) cotransport system that can be inhibited by the diuretic, furosemide (17). Measurement of the furosemide sensitive component of Na^+ influx in thalassemic erythrocytes showed it was normal in magnitude. A portion of the isotopic Na fluxes occurs by Na^+ exchange diffusion via a carrier that can also allow Li-Na countertransport (14). Taken together Na^+ exchange diffusion and ($\text{Na} + \text{K}$) cotransport account for some 40% of isotopic ^{22}Na influx in mature erythrocytes while the remainder is passive net leak. The net

leak of Na^+ into thalassemic erythrocytes from two patients was increased 2.3-fold above normal, whereas the same two patients had a corresponding 1.8- to 2.7-fold increase in erythrocyte ^{22}Na influx. Thus erythrocyte Na^+ permeability is increased in thalassemia major when it is measured either isotopically or by flame photometry.

Calcium uptake in thalassemic erythrocytes was greatly increased by 12-fold normal, although the relative increase was only 3.6-fold when compared with the control reticulocyte-rich cells. Calcium uptake was first measured in ATP-depleted cells, a condition which selectively inactivates the outward pumping of Ca^{2+} ions (12). Thalassemic cells pretreated with two different metabolic inhibitors (iodoacetate and iodoacetamide) each gave the same value for the subsequent uptake of Ca^{2+} and this suggests that Ca^{2+} entry resulted from ATP depletion and not from a membrane-perturbing effect of the inhibitor (10). The uptake of Ca^{2+} was then measured in cells depleted of Mg^{2+} since removal of this cation cofactor will also inhibit the calcium pump. Mg^{2+} -depleted cells did take up Ca^{2+} although the absolute uptake values were only 70% of that observed for ATP-depleted cells incubated in parallel flasks (Table III). Perhaps the lower Ca^{2+} accumulation by Mg^{2+} -depleted cells may be due to slight residual activity of the calcium pump since it was not possible to reduce Mg^{2+} below 0.02–0.05 $\mu\text{mol/ml}$ cells. The important finding was that Mg^{2+} -depleted thalassemic erythrocytes had a marked increase in Ca^{2+} uptake of the same relative amount as found previously for ATP-depleted cells i.e., sixfold above normal for patient C.C. In contrast neither ATP-depletion nor Mg^{2+} -depletion increased the isotopic Na^+ influx of normal or thalassemic erythrocytes. The above results make it likely that both depletion procedures inhibit the Ca^{2+} pump without increasing passive cation permeability and that the Ca^{2+} uptake by depleted cells reflects the true passive Ca^{2+} permeability of the intact nondepleted erythrocyte. The implications of this increased Ca^{2+} permeability are not clear. The capacity of the erythrocyte for outward Ca^{2+} pumping is very large (18–21) so that an increased Ca^{2+} leakiness of the thalassemic erythrocyte is likely to produce very little Ca^{2+} accumulation although even a slight increase may be significant in the etiology of the short erythrocyte survival found in this syndrome (22–24).

Why do thalassemic cells have a many fold increase in cation permeability? First, the permeability increase cannot be ascribed to an increased surface area to volume ratio of thalassemic cells, since this ratio was only increased by 15 to 25%. Second, it is clear that the increased permeability is not an immediate and direct consequence of i-antigen that is known to be present in thalassemia (3, 4), because erythrocytes from two persons with hereditary persistence of i-antigen had normal cation permeability. However the rare anomaly

of persistent i-antigen has a different genetic basis to thalassemia since in the former the i-antigen persists in the absence of I-antigen and moreover these persons are hematologically normal. Third, all the thalassemic patients who were studied showed 2.5 to 6.0% reticulocytosis but these cells were insufficient in numbers to account for the many fold increase in either cation permeability or cation pump numbers.

Other explanations must be considered for the increased Na^+ and Ca^{2+} permeability in thalassemia. A previous study has suggested that the increased K^+ permeability observed in thalassemic erythrocytes results from the interaction of free globin chains with the membrane (5). However, it is unlikely that free globin chains can account for the high Na^+ fluxes since the induction of Heinz bodies in erythrocytes by acetylphenylhydrazine has been shown to selectively increase K^+ permeability with little effect on Na^+ movements (25). Even when the K^+ permeability of thalassemia minor erythrocytes is increased following metabolic depletion no change in Na^+ permeability occurs (26). Moreover, globin chain precipitation in thalassemics cannot explain the observed increase in the density of cation pumps in the erythrocyte. Whether globin chain precipitation contributed to the high Na^+ influx in the single patient postsplenectomy (F.L.) also is uncertain since many erythroblasts were present in this cell suspension and it is likely that these cells have high cation permeability. Finally CDA type 2 erythrocytes which contain no free globin chains show increased Na^+ and Ca^{2+} fluxes of magnitude similar to thalassemia major.

The most likely explanation for the permeability changes derives from studies of the Ii-antigen system. Fetal erythrocytes possess i-antigen that is replaced after birth by the adult I-antigen. The finding of both i and I in adult thalassemic cells has led to the concept that the cell membrane is immature due to erythropoietic "stress" and skipped division of the marrow erythroblasts (3, 4). Immaturity of the cell membrane would also increase its cation permeability, a concept that has been documented for the human reticulocyte (10). Calcium uptake into thalassemic erythrocytes is increased by a mean 12-fold while Na^+ influx by only fourfold above normal values, so that thalassemic erythrocytes have an increased ratio of $\text{Ca}^{2+}:\text{Na}^+$ fluxes which is a feature of membrane immaturity also found in the human reticulocyte (10). Other evidence also supports the concept of membrane immaturity in thalassemic reticulocytes. Glucosamine is incorporated into the glycoprotein of reticulocyte stroma during short in vitro incubations and this incorporation is increased 5- to 10-fold in thalassemia over normal (27). However only those thalassemic patients showing evidence of erythropoietic "stress" (anemia; reticulocytes > 2%; increased i-titers) show an increased membrane permeability to cations. Thus hypertransfused

thalassemic patients with normal reticulocyte counts and no increase in i-titers have normal erythrocyte Na⁺ permeability.²

Finally it can be predicted that conditions associated with shortened marrow erythroid transit times and i-antigen on the erythrocyte will be associated with increased Na⁺, K⁺, and Ca²⁺ permeability. The present study shows an increased Na⁺ and Ca²⁺ permeability in CDA Type 2 erythrocytes with high titers of i-antigen obtained from a patient with a persistent anemia. Controversy exists over the etiology of increased Na⁺ and K⁺ fluxes and greater number of cation pumps per cell in Rh null disease (28, 29). Because this hemolytic condition is associated with increased titer of i-antigen, it seems likely that permeability changes result from membrane immaturity probably combined with the reticulocytosis (29). Thus a high cation permeability may be a marker of membrane immaturity not only in the thallemic syndromes, but in other conditions of erythropoietic stress.

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² Wiley, J. S., K. McCulloch, D. Ford, and R. Matthews. Unpublished observations.