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Research Article

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Potassium Permeability in β-Thalassemia Minor Red Blood Cells

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ABSTRACT The intracellular content of K^+ in thalassemia minor red blood cells is markedly reduced after incubation in autologous serum for 24 hr at 37°C. There is no compensatory increase in intracellular Na^+ concentration of the cell thus reduced. This change is due to an acquired increase in selective permeability of the membrane to K^+. This phenomenon follows the depletion of energy sources in the thalassemia minor cells but does not follow comparable depletion in normal cells. The loss of osmotically active intracellular contents probably accounts for the increased resistance of incubated thalassemia minor red blood cells to osmotic lysis.

INTRODUCTION

The thalassemia syndromes are a group of inherited disorders characterized by microcytic, hypochromic red blood cells (1). The homozygous state, thalassemia major, results in a severe anemia, and often death before puberty. The heterozygous state, thalassemia minor, is less severe and may be asymptomatic with little or no anemia. The underlying abnormality in the thalassemia syndromes is thought to be a defect in the production or in the control of the production of the globin chains of hemoglobin which results in a decreased concentration of complete hemoglobin molecules in the red cells. The disease is called β-thalassemia if β^A-chain production is decreased relative to α^A production and α-thalassemia if α^A-chain production is decreased relative to β^A. Although morphologic abnormalities of the membrane have been described in red cells from patients with thalassemia major, the surface texture of red cell membranes from thalassemia minor blood appears indistinguishable from normal (2).

The red cells from patients with either thalassemia major or thalassemia minor are resistant to in vitro osmotic lysis by hypotonic solutions of saline and become more resistant after incubation in autologous serum for 24 hr at 37°C. This resistance has been generally attributed to changes in cell shape or in intracellular protein osmotic pressure (3). While cells of patients with thalassemia major have elevated K^+ fluxes (4, 5), lose intracellular K^+, and decrease in size upon incubation (6), cells from patients with β-thalassemia minor have not been systematically investigated with regard to membrane function.

In order to examine the mechanism of increased resistance to osmotic lysis after incubation in the red cells of patients with β-thalassemia minor, we have investigated the changes in cation content and flux rate during incubation in autologous serum. We have found that there is a marked increase in permeability to K^+ with a consequent loss of cellular K^+, while there is no change in Na^+ permeability nor increase in cellular Na^+. The addition of excess glucose prevents both the increase in K^+ permeability and the fall in intracellular K^+. A reduction in cellular adenosine triphosphate (ATP) occurs in both normal and thalassemia minor cells; however, only the latter cells become permeable to K^+. Thus, thalassemia minor red blood cells appear to develop a membrane defect during incubation which results in a selective increase in K^+ permeability leading to a decrease in total cation content. The concomitant water loss and shrinkage of the red cells probably explains the increased resistance to osmotic lysis which has been described in these cells.

METHODS

Subjects. Red blood cells from patients with the heterozygous form of β-thalassemia (β-thalassemia minor) were studied (Table I). All patients exhibited some or all of the following features: red cell abnormalities characterized by hypochromia with microcytosis and poikilocytosis, minimal reduction in mean corpuscular hemoglobin concentration

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TABLE I

Clinical and Laboratory Data on 10 Patients with Thalassemia Minor

Patient	Hb	Retic.	MCV	MCHC	Hb A_2	Hb F	Family history	Ethnic origin	Palpable spleen	Other illnesses
	g/100 ml	%	μ^3	*g/100 ml*	%	%				
A. G.	8.8	5.4		28	5.1	0.8	yes	Negro	Splenectomy	Autoimmune hemolytic anemia
B. C.	11.2	1.2	85	31	3.0	0.4	?	English	no	
A. X.	14.3	1.2	67	30	5.7	0.5	yes	Greek	yes	
C. G.	9.9	2.8	60	29	5.6	1.0	yes	Greek E. Europe	no	
J. B.	9.4	1.2		26	5.4	2.6	yes	Scots-English	no	
L. B.	12.9	4.0	59	31	5.8	2.9	yes	Scots-English	no	
L. G.	9.1	3.0	68	27	5.4	1.1	yes	English	no	Hypogammaglobulinemia
K. L.	13.3	3.2		31	5.9	1.5	yes	English	yes	
T. L.	14.9	2.2	65	31	6.4	0.8	yes	English	no	
L. T.	11.0	1.0		30	6.3	0.5	yes	English	no	
Normal	12.0–15.0	0.5–1.5	82–92	32–36	2.5–3.5	0.8				

(MCHC),[1] reduction in mean corpuscular hemoglobin (MCH), and in mean corpuscular volume (MCV); adequate iron reserves demonstrated by bone marrow aspiration, serum iron, or by a history of adequate iron therapy without correction of the morphologic abnormalities of the red cells; increased percentage of Hb A_2 (7) or Hb F (8); increased resistance to osmotic lysis after 24 hr incubation at 37°C in autologous serum; palpable enlargement of the spleen; an appropriate family history of similarly affected individuals.

Normal red cells were obtained from healthy subjects.

Collection and preparation of blood. Blood was collected by venipuncture, defibrinated by the method of Dacie and Lewis (9) and transferred under sterile conditions in 2-ml portions to screw-capped tubes. Incubated samples were placed in a water bath at 37°C for varying periods. In experiments determining the effects of supplementary glucose, sterile 10% glucose solution was added to bring the final concentration to 10 mg/ml blood. In experiments determining the effect of ouabain, the drug was added to appropriate samples under sterile conditions to a final concentration of 10^{-4} M.

To determine cellular ATP or cation concentration, cells were separated from their plasma by centrifugation and washed three times in ice-cold 0.12 M $MgCl_2$. Samples of the packed cells were then removed for the appropriate determination.

Determination of osmotic fragility. The lysis of red cells in varying concentrations of saline was determined on defibrinated blood before and after a 24 hr in vitro incubation in autologous plasma at 37°C, according to the method described by Dacie and Lewis (9). The effect of the addition of glucose to the preincubate (10 mg/ml) on the subsequent osmotic fragility of the red cells was similarly evaluated. One vial of cyanomethemoglobinreagent (Hema-Cyn, Unitech) in 1 liter of a stock solution of phosphate-buffered NaCl (90 g/liter, pH 7.4) was used in preparing saline solutions of different concentrations. Hemolysis was allowed to proceed at room temperature for 20 min after which time the samples were centrifuged for 5 min and the optical density of the supernatant fluid at 540 mμ was determined with a Gilford microsample spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio, model 300N). The percentage of the cells hemolysed at each concentration of NaCl was calculated.

Determination of intracellular K^+ and Na^+ (10). A 0.1 ml sample of washed red cells was further washed four times in ice-cold 0.12 M $MgCl_2$. The washed cells were then hemolysed with a solution containing 0.0312 ml Non-Ion-Ox (A. S. Aloe Co., St. Louis, Mo.), 1.25 ml concentrated NH_4OH, and 0.004 moles CsCl per liter of solution. K^+ and Na^+ concentrations in these hemolysates were measured with an atomic absorption spectrometer (Perkin-Elmer Corp., Norwalk, Conn., model 303). The hemoglobin concentration of the hemolysates were measured by the method of Drabkin (11).

pH. The pH of the whole defibrinated blood was determined before and after incubation. In the samples of cells incubated in buffered medium, only the pH of the medium was measured.

Determination of red blood cell adenosine triphosphate. Determination of red blood cell adenosine triphosphate (ATP) was performed according to the method of Beutler and Baluda (12). Firefly extract and dessicated ATP were obtained from Sigma Chemical Co., St. Louis, Mo. Readings of light emission were made with a spectrofluorometer (Farrand Optical Co.) using Beckman 10-mm Pyrex cells (Beckman Instruments, Inc., Fullerton, Calif.). All readings were made at exactly 30 sec after addition of the cell hemolysate to the firefly extract. The ATP concentration was determined from a standard curve made at the same time with known quantities of ATP and the same firefly extract. Subsequent to these determinations, a systematic error in this method has been reported (13); however, this does not effect the comparisons between normal and β-thalassemia minor red blood cells made in this paper.

Influxes. K^{42} and Na^{22} influxes were measured in autologous serum in the presence of ouabain, 10^{-4} M final concentration using techniques previously reported (14). Briefly, 50-μl samples were taken at regular intervals into ice-cold polyethylene test tubes with 1.7 cc of dibutyl phthalate at the bottom and a layer of 8 cc of isotonic $MgCl_2$ on top. The tubes were immediately centrifuged, leaving the cell button below the dibutyl phthalate. The $MgCl_2$ was then removed and the top of the centrifuge tube washed once with isotonic $MgCl_2$ solution at room temperature which was then removed together with most of the dibutyl phtha-

[1] *Abbreviations used in this paper:* Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cells.

late leaving the cell button which was hemolyzed, and counted for radioactivity in a well-type gamma scintillation counter. The samples were diluted, centrifuged briefly to displace any suspended dibutyl phthalate and portions taken to determine hemoglobin, K^+, and Na^+. This method permits measurement of radioactivity uptake by cells without repeated washing of the cells or pipetting packed cells with a time resolution of 1–2 min.

Effluxes. ^{42}K efflux was measured by a rapid sampling method in thalassemia minor cells after incubation for 24 hr without glucose, because the influx was too rapid for accurate measurement by the method described above. The sampling time resolution was 2–3 sec. Efflux was determined by equilibrating the cells with ^{42}K for 2 hr at 37°C, after which they were packed in nylon tubes (3 × 70 mm), and the K^+ concentration of the supernate determined. The packed cells, isolated from the plasma by cutting the nylon tubes, were injected at time zero into a well-stirred pH-stated, thermostated medium containing the same K^+ concentration (50–60 mM) as the equilibrating medium, and $NaHCO_3$, 22 mM; $CaCl_2$, 1 mM; $MgCl_2$, 1.5 mM; and NaCl, 100–110 mM, sufficient to make the osmolarity 0.330–0.335 by freezing point depression against NaCl standards. This osmolarity and composition correspond to that of the extracellular phase after 24 hr of incubation without added glucose. The efflux was assumed to occur at potassium equilibrium and measured the steady state exchange of K^+ in these red cells. Serial samples of cell-free supernate were taken using a filtering technique of Dalmark and Wieth (15).

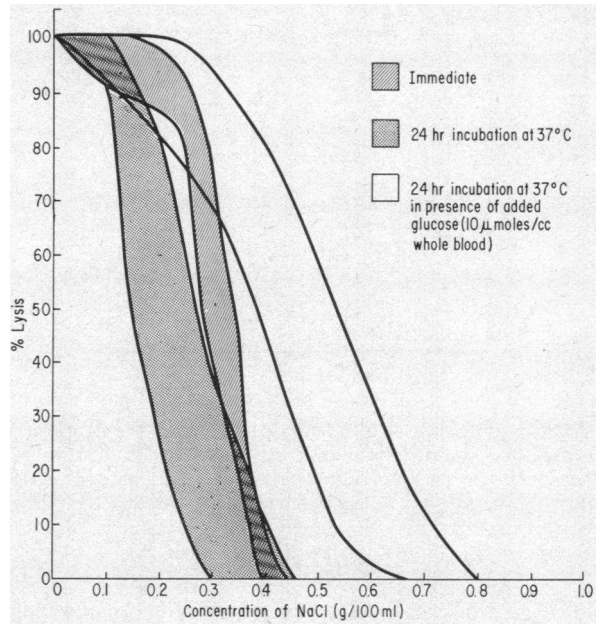
Calculations. The rate of potassium influx was calculated from the initial uptake of tracer over a 1-hr period at the external specific activity after 15 min preincubation with isotope. Back flux corrections is less than 2%.

The efflux is equal to the rate constant, $^{o}k_K^{LE}$ times the cellular K^+ concentration, where k is obtained from the slope of a plot of ln $(1 - [a(t)/a(\infty)])$ versus time, where a t and $a(\infty)$ are the supernatant radioactivity at the time, t and at equilibrium respectively. Strictly, this k should be corrected for the relative potassium pool sizes by the quotient of extracellular K^+ and total K^+; however, since the efflux suspension hematocrit is always less than 1% and extracellular K^+ concentration is the same order of magnitude as intracellular K^+ concentration the correction would be less than 1%. Therefore, no correction for potassium pool sizes is made.

The data is presented in terms of the rate coefficients, $^{i}k_K^{LE}$ and $^{i}k_{Na}^{LE}$. The subscript denotes the ion species, the left superscript, i or o, denotes influx or outflux, the right superscript, LE, denotes tracer leak and exchange in the presence of ouabain. The rate coefficient is in reciprocal hours though more precisely it is the flux in (mmoles/[liter cell × hour]) divided by the medium ion concentration, in millimoles per liter of supernate in the case of influx or the flux divided by the ion concentration in the cell, in millimoles per liter cell water in the case of an efflux. The permeability coefficient of the cell membrane, $P = kV/Af_\psi$, requires a knowledge of the cell volume (V), area (A), and electrical potential (ψ) ($f_\psi = [\psi F/RT]/(1 - \exp[-\psi F/RT])$ [16]), which were not determined and cannot be assumed to be constant over the course of the incubation.

RESULTS

Osmotic fragility of normal and thalassemia minor red blood cells. Osmotic fragility curves of normal and thalassemia minor red blood cells are shown in Fig. 1. In



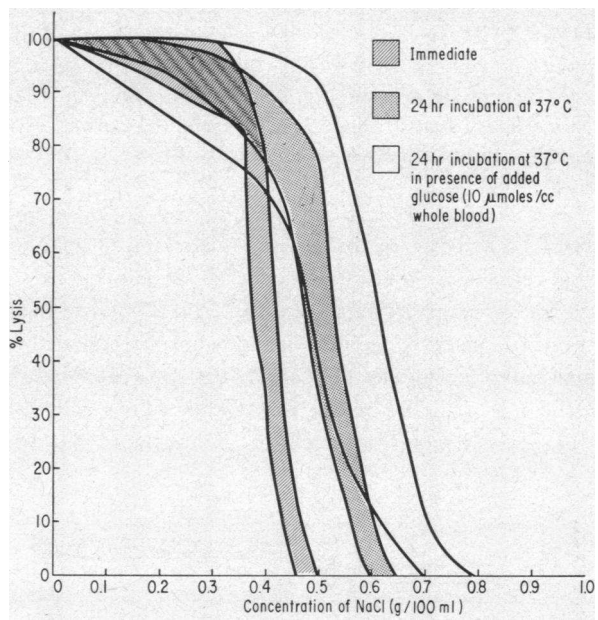


FIGURE 1a The osmotic fragility of red cells of patients with thalassemia minor. The range shown is ±1 standard deviation from the mean at each concentration of NaCl tested.

FIGURE 1b Osmotic fragility curves of seven normal donors graphed as in Fig. 1a.

fresh defibrinated blood, 50% of thalassemia minor cells lysed at concentrations of NaCl ranging from 0.25 to 0.38 g/100 ml, whereas 50% of normal cells lysed at concentrations ranging from 0.38 to 0.43 g/100 ml. After incubation for 24 hr at 37°C, the osmotic fragility of normal cells was increased (50% lysis at 0.48–0.53 g/100

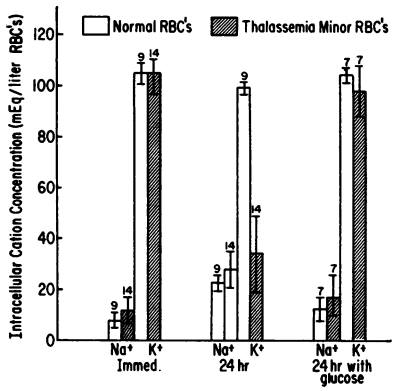


FIGURE 2 The intracellular Na^+ and K^+ concentration of normal and thalassemia minor red blood cells before and after incubation in autologous serum with and without added glucose. The height of each bar corresponds to the mean, the standard deviation is shown in the bracketed bar, and the number of samples is shown above each bar.

ml NaCl) whereas thalassemia minor cells became more resistant to osmotic lysis (50% lysis as 0.15–0.33 g/100 ml NaCl). However, in the presence of added glucose during incubation, thalassemia minor red cells became more susceptible to osmotic lysis (50% lysis at 0.41–0.58 g/100 ml NaCl), and closely resembled normal cells similarly treated (50% lysis at 0.48–0.60 g/100 ml NaCl).

Intracellular content of K^+ and Na^+. Intracellular content of K^+ and Na^+ of thalassemia minor and normal red blood cells before and after incubation in autologous serum for 24 hr at 37°C is shown in Fig. 2. Before incubation, the mean K^+ content in both normal and thalassemia minor cells was the same (104 mEq/liter cells). At the end of the 24 hr, the thalassemia minor cells had a markedly reduced intracellular K^+ content (34 ±15 mEq/liter original cells [2]) whereas normal cells had lost virtually no K^+ and had a final content of 99 ±3 mEq/liter cells [2] after incubation. The intracellular Na^+ content before incubation was 8 ±3 mEq/liter cells for normal cells and 12 ±4 mEq/liter cells for thalassemic cells. There was essentially no difference in the amount of Na^+ accumulated by normal and thalassemia minor cells during the incubation period. Normal red cells increased their total cation ($Na^+ + K^+$) content by 10 mEq/liter cells during incubation, while thalassemia minor red cells had a net loss of 55 mEq of total cation ($Na^+ + K^+$) liter cells.

For both normal and thalassemia minor cells, the addition of glucose to the medium before incubation prevented any appreciable change in total cation content over the 24 hr period.

The time course of net K^+ loss from thalassemia minor red cells was determined by measuring intracellular cation content at intervals during the incubation period (Fig. 3). No net loss of K^+ was observed until approximately 10 hr of incubation. During the next 14 hr, the net K^+ content gradually decreased.

The effect of ouabain on intracellular cation content. Fig. 4 shows the changes in cell K^+ and Na^+ content in the presence and absence of 10^{-4} ouabain during the incubation. The total sodium uptake of thalassemia minor red cells in the absence and presence of ouabain was similar to that in comparably treated normal red cells.

While the net sodium uptake of thalassemia minor and normal cells in the presence and absence of ouabain are similar to each other as well as to data in the literature (17), the net K loss of thalassemia minor cells is greatly

[2] Mean ±1 standard deviation.

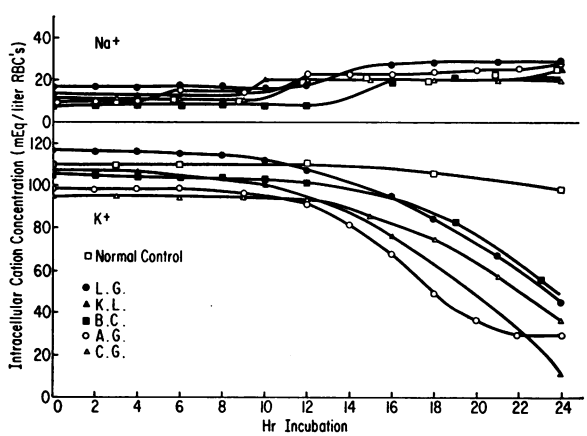


FIGURE 3 The rate of change of intracellular cation concentration of cells from five patients with thalassemia minor and one normal donor during incubation in autologous serum.

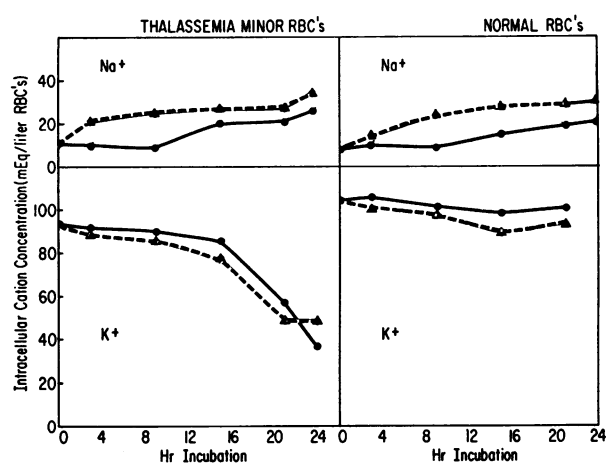


FIGURE 4 The changes in intracellular cation concentration after incubation in the absence (circles and solid lines) or presence (triangles and interrupted lines) of 10^{-4} molecular ouabain.

accelerated only after 12 hr of incubation. For the 1st 12 hr, the rates of K loss are the same in the two types of cells. After 12 hr the thalassemic minor cells lose 5–6 mM K^+/liter cell per hr in the absence or presence of ouabain while the normal cells lose K at about 0.5 mM K^+/liter original cells per hr only in the presence of ouabain.

Decrease of ATP in normal and thalassemic washed red cells. At the onset of incubation the mean level of cellular ATP in normal cells was higher than in thalassemia minor cells. ATP, levels decreased at the same rate in both normal and thalassemia minor cells and by 16 hr, the level was less than 5% of the initial value (Fig. 5).

pH. The pH of the samples of whole blood was initially between 7.25 and 7.50. With 10 hr, the pH of both normal and thalassemia minor red cell suspensions had fallen to 7.0–7.1 and maintained this level throughout the remainder of the incubation period.

Na^+ and K^+ fluxes. The results of the tracer influx measurements experiments are given in Tables II and III. Most prominent is the increase in potassium leak of β-thalassemia trait cells after 24 hr of incubation in the absence of added glucose. The influx measurements were inadequate to determine this rapid flux due to the limited time resolution of cell separation by centrifugation. Using a rapid filtration technique, the efflux of ^{42}K from cells presumed to be in the steady state with respect to total potassium has two components each representing approximately half of the cell potassium. From four experiments the average rapid component has a half-time of 1.6 min which is equivalent to ${}^0k_K^{LE} = 25.2$ hr^{-1}. The slower component has a half-time of 14.3 min or ${}^0k_K^{LE} = 2.9$ hr^{-1}. Whether these two components represent physiologic compartments or cell populations or changing physical parameters of the system during the efflux (non–steady state) cannot be determined from this data.

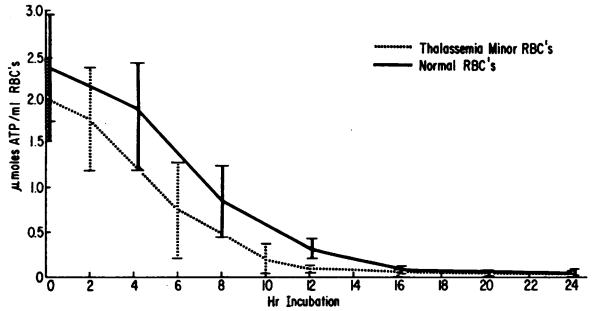


FIGURE 5 Change in cellular adenosine triphosphate (ATP) concentration of normal and thalassemia minor red blood cells during incubation in autologous serum. The mean of six thalassemia minor samples and five normal samples are shown; the one standard deviation from the mean is shown by the vertical bars. The zero time values here agree with those of Beutler and Baluda who developed the method of ATP analysis used in this study. However, these values are systematically 140% of the true value probably due to the presence of protein in the red cell extracts and its absence in the standard ATP solutions (Beutler and Mathai [13]). The conclusions that thalassemia minor RBC's have normal ATP levels and the time course of ATP depletion is the same as normals when incubated without glucose, are not altered by the systematic error in ATP levels in these analyses.

While the mean potassium influx rate coefficients are different for fresh normal and fresh thalassemia minor cells, both values are within the ranges of normal cells previously reported (18–20). Sodium rate coefficients are not different in the two types of cells. Incubation for 24 hr in the presence of glucose decreases ${}^1k_K^{LE}$ and ${}^1k_{Na}^{LE}$, meaning that the cells are more impermeable to these ions. In the cells incubated without glucose ${}^1k_{Na}^{LE}$ is only slightly elevated after 24 hr incubation while ${}^1k_K^{LE}$ is increased by at least 100-fold.

TABLE II

The Rate Coefficients for Potassium Influx, ${}^ik_K^{LE}$, in the Presence of 10^{-4} Ouabain in Normal and Thalassemia Minor Red Cells before and after 24 hr Incubation at 37°C

	${}^ik_K^{LE}$ (hr^{-1})	
	Normal RBC	Thalassemia minor RBC
Fresh red cells		
With or without added glucose	0.074 ±0.10 n = 8	0.055 ±0.012 n = 19
Incubated red cells	(range)	
With added glucose	0.068 (0.065–0.070), n = 3	0.045 ±0.025, n = 16
No added glucose	0.037 (0.036–0.039), n = 2	>1.8–4.9*, n = 16

* These values are lower bounds due to limitations of sampling rate in influx experiments. Rapid efflux measurements reveal two components, the fast component ${}^0k_K^{LE} = 25.2$ hr^{-1}, the slower component ${}^0k_K^{LE} = 2.9$ hr^{-1}.

TABLE III

The Rate Coefficients for Sodium Influx, ${}^{i}k_{Na}^{LE}$, in the Presence of 10^{-4} M Ouabain in Normal and Thalassemia Minor Red Cells before and after 24 hr Incubation at 37°C

	${}^{i}k_{Na}^{LE}$ (hr^{-1})	
	Normal RBC	Thalassemia minor RBC
Fresh red cells		
With or without added glucose	0.020	0.022 ±0.005 n = 8
Incubated red cells		
With added glucose	0.010	0.010 (0.008–0.013), n = 4
No added glucose	0.021	0.028 (0.028–0.029), n = 3

DISCUSSION

These results clearly indicate that when red blood cells of patients with β-thalassemia minor are incubated in autologous serum, their intracellular K^+ content decreases (6) for thalassemia major cells, whereas intracellular Na^+ content increases only slightly. These changes result in a net reduction in total intracellular alkali metal cations ($Na^+ + K^+$). The intracellular K^+ content of normal cells incubated under the same conditions, in contrast, decreases only slightly and the total cation markedly after 10–12 hr, as had been noted by Selwyn content is, in fact, slightly increased.

This depletion of intracellular ionic contents is the probable explanation of the abnormal resistance of incubated thalassemia minor cells to osmotic lysis. Red cell resistance to osmotic lysis is correlated with the ratio of surface area to osmotically active cell contents; an increase in the numerator or a decrease in the denominator results in the ability of the cell to withstand greater osmotic stress. While it is unlikely that the membrane surface area of thalassemia minor cells increases during incubation, the loss of intracellular K^+ probably explains the marked increase in osmotic resistance noted in these cells after incubation.

The net fall in intracellular K^+ in incubated thalassemia minor red blood cells may be attributed to a reduced influx and/or an increased efflux of the cation. The tracer K^+ influx is elevated in these cells, however this may be in part K^+ exchange diffusion. If net K loss of 5–6 mmoles/liter cell per hr were due to decreased influx, the tracer influx must be at least this value before it is reduced. This is not so. Therefore, we may conclude that increased efflux due to a selective increase in passive K permeability must be responsible for the large net K loss that is observed. This conclusion is confirmed by the large ouabain-insensitive K influx and efflux after 24 hr incubation.

Since the active transport of Na and K in normal cells only accounts for 15–20% of the ATP utilization there may be significant differences in active transport during incubation while the rate of total ATP depletion is the same in the two types of cells.

The selective permeability to K^+ is acquired during the course of the incubation. Depletion of energy sources appears to be a prerequisite for the appearance of this membrane lesion. The net loss of K^+ is not seen until after the cellular ATP concentration is very low. Glucose, when added at the beginning of the incubation period, prevents the development of the selective K^+ permeability. Cell metabolism has been shown to play a role in K^+ permeability in red cell membranes (21) and may play a mediating role in the increase K^+ permeability of these cells. Inhibition of glycolysis or substrate deprivation appears necessary for the action of fluoride on passive K^+ permeability (22, 23). Gardos (24) on the other hand has demonstrated that the addition of calcium chelators (EDTA) or the removal of calcium from the system will prevent the fluoride-induced K^+ permeability, independent of fluoride effects on metabolism or ATP levels. Romero and Whittam (25) have shown that an elevated internal calcium concentration can be correlated with increased potassium permeability and that ATP depletion promotes an increased intracellular calcium. The increased potassium fluxes they observed are much less than those found here in incubated thalassemia minor red cells, and whereas increased intracellular calcium causes an increased sodium permeability, no change in the sodium rate coefficient was found in this study.

The changes in pH would not appear to account for the development of selective K^+ permeability in thalassemia minor. The change in pH during incubation was the same for normal and thalassemia minor cells but only the latter developed the change in K^+ permeability. Flynn and Maizels have shown that pH changes between 6.85 and 7.65 do not alter the permeability characteristics of normal cells (26). However, the possibility exists that part of the underlying mechanism for in-

creased potassium permeability may be due to an enhanced sensitivity to the pH changes.

The increase in potassium leakage in thalassemia minor cells caused by incubation for 24 hr at 37°C is larger than other specific increases in potassium permeability. Wilbrandt (27) and Passow (21) demonstrated that NaF in high concentrations (10^{-2} M) and lead in low concentrations (10^{-7} moles/g cells) can cause a selective increase in the K^+ rate coefficient by a factor of 20. The enhancement of net K^+ leakage in sheep red cells treated with valinomycin (10^{-7} M) found by Tosteson, Cook, Andreoli, and Tieffenberg (10) is nearly 30-fold. Treatment of human red cells with butanol (0.3 M) specifically increases K^+ leakage 150-fold (28). The specific increase in ${}^{\circ}k_K^{LE}$ reported here is more than a 1200-fold enhancement. This was calculated by comparing the normal efflux rate coefficient 0.02 hr^{-1} (20) with ${}^{\circ}k_K^{LE} = 25.2$ hr^{-1} for the fast component of the efflux. Since only a lower bound on the influx rate can be measured for incubated thalassemia minor cells, we can only state that the K^+ influx rate coefficient increased more than 100-fold. Making the assumption that the cells are in steady state with respect to K^+ and calculating the expected influx rate coefficient from the faster efflux rate coefficient one estimates ${}^{i}k_K^{LE}$ to be approximately 20 hr^{-1} or increased by 400-fold.

The ability of chemical agents to induce a K^+ leakage without altering Na^+ leakage and the ability of the red cell membrane in thalassemia minor to undergo an even greater specific increase in K^+ leakage, suggest that there are separate rate-limiting control sites in the red cell membrane for passive penetration of K^+ and for Na^+ passive penetration. There appear to be two classes of agents which increase passive red cell membrane permeability to both Na^+ and K^+: reversible agents which can be eluted from the cell to restore normal permeability (29, 14) and irreversible agents which may form covalent bonds with some membrane component (30, 31). The fact that a single agent can effect the permeability of both cations only implies that the rate-controlling sites for Na^+ and K^+ permeability have a similar reactivity and not that they are a single population of sites with each site controlling both ion permeabilities. In the membrane of red cells from patients with thalassemia minor, the rate-controlling sites for potassium are more sensitive to the stress of substrate depletion or its consequence than are the rate controlling sites for Na^+. They are also more sensitive to the stress of substrate depletion than the rate-controlling sites for K^+ or Na^+ in normal red cell membranes.

The selective permeability to K^+ observed in incubated thalassemia minor red cells is the first demonstration of such a defect in human cells not treated with chemicals. The present data offer no basis for speculation as to the mechanism of this change in thalassemia minor but do demonstrate that the membrane in these cells is functionally different from that of normal cells. The relationship of this difference to the consequences of the defect in globin chain production in these cells remains to be investigated. Further characterization of the highly permeant, depleted, thalassemia minor red cells with regard to calcium sensitivity and the nature of the two components of the potassium efflux are in progress.

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