Increased Calcium Permeability of Cold-Stored Erythrocytes

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The calcium, sodium, and magnesium permeability of erythrocytes from blood stored at 4°C in various anticoagulant media has been studied and compared to that of fresh erythrocytes. Passive influx of Ca\(^{2+}\) was measured at 37°C in cells pretreated to abolish Ca\(^{2+}\) pumping and was up to 5-fold greater for cold-stored erythrocytes than for fresh cells. The Ca\(^{2+}\) leakiness developed gradually after day 2 and reached a maximum by day 7 of cold storage in ACD. CPD, CPD-adenine, or heparin anticoagulants. The total calcium content of cold-stored erythrocytes in ACD was not significantly different from that of fresh erythrocytes. However, when cold-stored erythrocytes were reincubated at 37°C in media containing 1.5 mM ionized calcium and substrates to regenerate ATP, a net gain of Ca\(^{2+}\) occurred that was greater for stored than for fresh erythrocytes. Cold storage of blood for up to 6 wk in any anticoagulant did not alter either sodium or magnesium permeability.

Red cell ATP was also measured and fell steadily during cold storage in ACD or CPD, but more slowly in CPD-adenine medium. Since a several-fold increase in Ca\(^{2+}\) permeability preceded any significant change in red cell ATP, it is likely that a selective calcium leak develops independently of the fall in ATP concentration that occurs on cold storage.

Storage of Blood at 4°C in Anticoagulant, such as acid citrate dextrose (ACD), leads to a progressive increase in the proportion of nonviable red cells that are removed within the 24-hr period following transfusion.\(^1,2\) This loss of cell viability has been attributed to the fall in red cell adenosine triphosphate (ATP) concentration that occurs during cold storage.\(^3,5\) Other evidence suggests that a progressive and irreversible membrane deterioration limits the viability of cold-stored blood. Red cells have been shown to lose about 25% of their lipid over 6 wk of cold storage, and this loss of membrane material is accompanied by an increase in median corpuscular fragility.\(^6\) What factors control this progressive reduction in red cell membrane material are currently unknown.

In this study, the Na\(^{+}\), Mg\(^{2+}\) and Ca\(^{2+}\) permeability of red cells from blood stored up to 6 wk at 4°C in various anticoagulant media has been measured. The results show a several-fold increase in Ca\(^{2+}\) permeability after as little as 3-day storage, with no change in the permeability to other cations. This marked increase in Ca\(^{2+}\) permeability preceded any fall in ATP concentration of the stored cells.

MATERIALS AND METHODS

Storage and Preparation of Erythrocytes

Healthy young volunteers were selected as donors on the basis of normal blood counts and a reticulocyte count of less than 1.5%. None had donated blood in the previous 4 mo. Venous blood from the one donor was drawn into sterile plastic bags (Fenwal Incorporated) containing either ACD, citrate phosphate dextrose (CPD), or CPD plus adenine (0.4 mM final concentration) anticoagulant media in a ratio of 150 ml blood to 20 ml anticoagulant, and the bag was stored at 4 ± 1°C. In experiments comparing the different preservatives, the blood from one donor was immediately divided into the three separate bags. Storage in heparin was achieved by adding 80 ml blood to 2 ml anticoagulant containing heparin (2000 U), glucose (0.5 g), and 0.8 M imidazole Cl, pH 6.0. The pH of the heparinized blood was 7.23 compared with 7.27 for CPD-blood and 7.08 for ACD-blood at room temperature. At intervals, each bag was mixed, 30 ml of blood was aseptically sampled, and the erythrocytes were separated by washing 3 times in a medium of composition 145 mM NaCl, 5 mM KCl plus 20 mM imidazole Cl, pH 7.4, with careful removal of buffy coat after each wash.

Total Cell Calcium Concentration

Erythrocytes were washed free of buffy coat and further washed four times at 4°C in 150 mM NaCl from which Ca\(^{2+}\) contaminant had been removed by filtration through a Chelex-100 ion exchange resin (Bio-Rad Laboratories, Calif.). Cells were hemolyzed in 0.01 N NH₄OH, the stroma were dissolved by drop-wise addition of 10% Tergitol NP-10, and calcium in the hemolyzate measured by atomic absorption spectroscopy at 422.6 nm with acetylene fuel plus nitrous oxide with 5 mM LiCl added to suppress ionization.\(^7\)

Calcium Uptake by ATP-Replete Cells

Venous blood, either drawn freshly or cold-stored in ACD for 3–4 wk, was washed 3 times in medium containing 145 mM NaCl, 5 mM KCl plus 20 mM imidazole Cl, pH 7.4, to remove buffy coat and suspended at 40% hematocrit in medium of composition 145 mM NaCl, 5 mM KCl, 20 mM imidazole Cl, pH 7.4, plus 10 mM glucose. The suspension was warmed to 37°C, a sample removed for analysis of total cell calcium concentration, and \(^{45}\)CaCl₂ added to give a final concentration of 1.5 mM Ca and 1 μCi/ml. After 60-min incubation, the cells were analyzed both for...
total cell calcium as well as for isotopic $^{45}\text{Ca}^{2+}$ uptake (see below).

**Red Cell ATP Depletion**

Most influx measurements were performed on washed erythrocytes depleted of ATP by preincubation at 30% hematocrit for 90 min in medium plus 1 mM iodoacetate and 10 mM inosine. The erythrocytes were then washed twice to remove inhibitor.

**Unidirectional Calcium Influx**

Washed erythrocytes were added to prewarmed medium of composition 145 mM NaCl, 5 mM KCl, 20 mM imidazole Cl, pH 7.4, plus 1.5 mM $^{45}\text{CaCl}_2$ (1 $\mu$Ci/ml) at a final hematocrit of 30%. Samples were taken after 15 min and after 1, 2, 3, and 4 hr, and washed 4 times in ice-cold 150 mM NaCl. Each pellet was hemolyzed in 0.01 N NH$_4$OH, and part of the hemolysate was deproteinized with 6% (w/v) perchloric acid, and $^{45}\text{Ca}^{2+}$ 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 fresh and stored erythrocytes.

In some experiments, Ca$^{2+}$ influx was measured into washed erythrocytes depleted of Mg$^{2+}$ by a 37°C preincubation in the dark for 2 hr at 2% hematocrit in the above washing medium plus 10 mM glucose, 2 mM EDTA, and 17.5 nmole/ml cells of ionophore A23187 (Eli Lilly & Co., Ind.)$^9$ The erythrocytes were then washed 5 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 4 more times at room temperature in a medium comprised of 145 mM NaCl, 5 mM KCl, and 20 mM imidazole Cl, pH 7.4. The cellular Mg$^{2+}$ concentration was determined by atomic absorption spectrophotometry and was within the range of 0.02-0.05 μmole Mg$^{2+}$/ml cells. $^{45}\text{Ca}^{2+}$ uptake was measured on the washed Mg$^{2+}$-depleted erythrocytes as described above except for the addition of 5 mM inosine to the $^{45}\text{Ca}^{2+}$-containing medium.

**Mg$^{2+}$ Uptake**

Freshly washed cells and cells depleted of Mg$^{2+}$ by the above-described process were incubated in medium containing 110 mM MgCl$_2$, 10 mM glucose, plus 20 mM imidazole Cl, pH 7.4, at 37°C for 4 hr. They were then rapidly washed 4 times in ice-cold 150 mM NaCl. Each pellet was hemolyzed, and internal Mg$^{2+}$ concentration was determined by atomic absorption spectrophotometry.

**$^{22}\text{Na}^{+}$ Influx**

Washed cells were incubated at 37°C for 10 and 20 min in saline medium plus $^{22}\text{NaCl}$ (1 μCi/ml), and $^{22}\text{Na}^{+}$ influx was measured as described previously.$^9$ Media always contained 10 mM glucose plus 50 μM ouabain.

**Red Cell ATP Concentration**

Whole blood of known hematocrit was deproteinized with 2.5 vol of 6% (w/v) perchloric acid, centrifuged at 4°C and the supernatant neutralized with 5 M K$_2$CO$_3$. ATP in the extract was estimated spectrophotometrically by measuring the reduction of NADP$^+$ by a coupled hexokinase–glucose-6-phosphate dehydrogenase reaction.$^11$

**Complement Coating of Red Cells**

Normal fresh erythrocytes were washed and ATP depleted as described above. The cells were then washed 0.4 vol cells incubated in 0.3 vol of ABO-compatible serum containing high titer cold agglutinin (anti-I) plus 0.3 vol of autologous serum to provide complement components. After 15 min at 4°C, the cells were warmed to 37°C for another 15 min and then washed 6 times in saline at 37°C. Complement coating (C3 and C4) on these cells as well as on cold-stored cells was analyzed by a tube Coombs technique with three different anti-C3 and anti-C4 reagents (Behringwerke, West Germany; CSL, Australia; and Ortho Diagnostics, Raritan, N.J.) and visual agglutination in tubes scored on a 0-4+ basis.

**Statistics**

Mean values ± 1 SD are shown unless otherwise noted, and differences between sample means were analyzed by a t-test.

**RESULTS**

**Calcium Concentration of Stored Erythrocytes**

The mean calcium content of fresh erythrocytes from 7 normal donors was 4.3 ± 1.9 nmole/ml cells after 4 washes in a verified Ca$^{2+}$-free medium at 4°C. The calcium content of erythrocytes cold-stored for 3–4 wk in ACD anticoagulant was 3.2 ± 1.0 nmole/ml cells ($n = 7$), which was not significantly different from fresh cells.

**Net Calcium Gain on Reincubation of Stored Erythrocytes**

To find if cold-stored cells gain Ca$^{2+}$ when exposed to physiologic levels of this cation, the cells were incubated with glucose for 1 hr at 37°C in media containing 1.5 mM ionized $^{45}\text{Ca}^{2+}$ and were then washed for Ca$^{2+}$ determination. Fresh cells gained calcium and the increment was $1.7 ± 2.6$ nmole/ml cells measured by atomic absorption spectroscopy or 1.9 ± 1.0 nmole/ml cells measured by the uptake of isotopic calcium into the same cells. In contrast, the cells cold-stored for 3–4 wk in ACD gained even more Ca$^{2+}$ at 37°C and the increment was 3.7 ± 1.6 nmole/ml cells measured by atomic absorption spectroscopy or 3.3 ± 2.0 nmole/ml cells measured by simultaneous isotopic $^{45}\text{Ca}^{2+}$ uptake. Thus, an increase in cell calcium can be shown either by flame photometry or isotopic techniques, and this gain of calcium was
significantly greater for cold-stored than for fresh cells ($p < 0.02$). Nevertheless, the increment in cell calcium is very small because it represents the net balance between passive influx and outward pumping of this cation. During the above incubation of cold-stored cells with glucose, the monovalent cation content of the cells was partially restored towards normal levels with cell K' increasing by about 1 μeq/ml cells/hr and with cell Na' decreasing by a reciprocal amount. The presence of as much as 10 mM Ca^{2+} in the incubation medium had no significant effect on this rise in cellular K' concentrations.

**Calcium Permeability of Stored Erythrocytes**

The unidirectional passive influx of Ca^{2+} was then measured in two ways. In the first method, cells were depleted of ATP to inhibit outward pumping of Ca^{2+} and then incubated in a 1.5-mM ^{45}Ca-medium. There was a rapid initial uptake of isotopic ^{45}Ca^{2+}, as well as a slower uptake that was linear between 60 min and 4 hr and both components of uptake were increased fivefold in the cold-stored compared to fresh erythrocytes (Fig. 1). In the second method, cells were depleted of Mg^{2+} using ionophore A23187, a procedure that again inhibits the pump by removal of a different cofactor. The depleted cells took up ^{45}Ca^{2+} from a 1.5 mM ^{45}Ca-medium and uptake was the same for Mg^{2+}-depleted as for ATP-depleted cells from the one donor. This second approach again demonstrated a five-fold greater ^{45}Ca^{2+} uptake in cold-stored cells than in fresh erythrocytes (Fig. 1).

**Sodium and Magnesium Permeability of Stored Erythrocytes**

Despite the marked increase in Ca^{2+} permeability, no significant changes in either ^{22}Na' or Mg^{2+} influxes were found in cold-stored erythrocytes. Red cell ^{22}Na' influx was measured on blood sampled at intervals up to 6 wk of cold storage, but all influxes fell within the range for normal fresh erythrocytes (Table 1). Moreover, identical values for red cell ^{22}Na' influx were obtained for blood from the one donor stored in three different anticoagulant media (Table 1). The Mg^{2+} content of fresh and cold-stored erythrocytes was identical (2.0 ± 0.14 and 2.1 ± 0.15 μeq/ml cells, respectively), and because of the low permeability of cells to this cation, it was not possible to reliably measure Mg^{2+} uptake in short-term incubations. However, the Mg^{2+} uptake into erythrocytes depleted of Mg^{2+} could be accurately quantitated after a 4-hr incubation of cells in 110 mM MgCl₂ medium, pH 7.4, at 37°C. The Mg^{2+} permeability of fresh human erythrocytes measured by the uptake of this cation was extremely low (0.06 μmole/ml cells/hr) and was not increased in red cells after 3–5 wk of cold storage (Table 1).
Calcium Permeability in Different Anticoagulants

Blood from each of several donors was drawn into three different anticoagulant media (ACD, CPD, CPD-adenine) and stored identically at 4°C. Serial permeability measurements performed over 6 wk of storage showed that Ca²⁺ leakiness developed gradually between days 2 and 7 of storage (Fig. 2). The rise in Ca²⁺ permeability occurred sooner in cells stored in acidic than in the neutral anticoagulants, since the Ca²⁺ uptake of ACD-stored cells at day 4 was greater than for cells stored in CPD or CPD-adenine. However, by day 7, the Ca²⁺ permeability had reached a maximum of 4-5-fold the value for fresh erythrocytes, and no differences were apparent between the three anticoagulants used. Blood stored in a different neutral anticoagulant, heparin (25 U/ml), showed a similar increase in red cell Ca²⁺ permeability over 1 wk of cold storage, as was found for CPD anticoagulant. A significant decline in the Ca²⁺ permeability of cells stored in CPD-adenine was observed at 4, 5, and 6 wk of cold storage (p < 0.02) compared to cells stored in either ACD or CPD anticoagulant.

Calcium Permeability of Complement-Coated Erythrocytes

Increasing amounts of the third component of complement (C3) have been reported on the red cell membrane during cold storage. Fresh erythrocytes were coated with complement and their Ca²⁺ influx was compared to noncoated cells. Attachment of C3 plus C4 to ATP-depleted red cells increased the ⁴⁵Ca²⁺ influx from 20% to 45% at all incubation times between 15 min and 4 hr. This increase in Ca²⁺ influx was only observed with heavy complement coating of cells showing agglutination scores of 3+ for both anti-C3 and anti-C4 reagents. In contrast, cold-stored cells not treated with cold agglutinin showed undetectable complement reactions with a manual tube Coombs technique.

Comparison of Ca²⁺ Permeability to Red Cell ATP During Storage

Since a fall in red cell ATP is considered a factor in the "storage lesion," the red cell Ca²⁺ permeability and ATP concentration were compared during cold storage of cells obtained from the same donor. Red cell ATP fell steadily during cold storage in ACD or CPD from an initial 1.1 μmole/ml cells to values of 50% normal by 21 days and 20% normal by 42 days. Storage of blood in CPD-adenine significantly retarded the fall in red cell ATP. No fall in ATP occurred in the first week of storage in CPD-adenine, while at all subsequent periods of cold storage between 1 and 6 wk, ATP was higher in CPD-adenine than in either ACD or CPD (p < 0.02). A many-fold increase in Ca²⁺ permeability was already established by day 7 of storage in all anticoagulant media, a change that preceded any significant fall in red cell ATP content.

DISCUSSION

An increase in red cell Ca²⁺ permeability is an early and constant finding following storage of human blood at 4°C in any anticoagulant. Red cell passive Ca²⁺ leak has been previously reported to be increased in blood stored in ACD anticoagulant at 4°C, although the specificity of this leak was unclear, since fluxes of other cations were not measured. In this study, the isotopic Na⁺ influx was unchanged throughout cold storage (Table 1), which indicates that the red cell membrane maintains a constant permeability to monovalent cations. Erythrocytes exhibit a very low permeability to magnesium as measured with ²⁵Mg tracer. Indeed the Mg²⁺ permeability was found to be so low that it could only be measured in cells that had been depleted of Mg²⁺ and then incubated in isotonic 110 mM MgCl₂ (Table 1). This large inward concentration gradient produced a net Mg²⁺ uptake in fresh washed erythrocytes of 0.24-0.32 μmole/ml cells over a 4-hr incubation at 37°C, and since there are no pumps for Mg²⁺-ions, this uptake measures the passive permeability of the membrane. No change in Mg²⁺ permeability was found in cold-stored cells, so that the increase in Ca²⁺ leak seems specific for this cation.

Membrane Ca²⁺ permeability is also low and represents a physiologic specialization of red cells that is selectively acquired during maturation of the reticuloocyte to the red cell. Measurement of passive inward Ca²⁺ permeability, however, is complicated by the
presence of a pump that has a large capacity for outward \( \text{Ca}^{2+} \) pumping as well as high affinity for internal \( \text{Ca}^{2+} \) ions.\(^{16-19} \) In the present study the red cell \( \text{Ca}^{2+} \) permeability was measured by the unidirectional \( { }^{45}\text{Ca}^{2+} \) influx under conditions in which the outward \( \text{Ca}^{2+} \) pump was inhibited. Inhibition was achieved either by depletion of ATP or of \( \text{Mg}^{2+} \) ions, both of which are cofactors for the pump. Since depletion of either cofactor resulted in identical values for red cell \( { }^{45}\text{Ca}^{2+} \) uptake (Fig. 1), it is likely that this uptake reflects the passive \( \text{Ca}^{2+} \) permeability of the intact nondepleted erythrocyte. An analogous approach was taken by Szasz et al.\(^{14} \) who studied \( \text{Ca}^{2+} \) influx in the presence of 0.2 mM lanthanum to block the \( \text{Ca}^{2+} \) pump. This cation has other effects, however, such as inducing red cell agglutination and inhibiting the passive influx of \( \text{Ca}^{2+} \) ions through membranes.\(^{20,21} \) Thus, the absolute values for \( \text{Ca}^{2+} \) influx in the presence of \( \text{La}^{3+} \) ions cannot be compared with values in the present study.

The main finding shown in Fig. 2 is that the \( \text{Ca}^{2+} \) permeability of erythrocytes increases several-fold between 2 and 7 days of cold storage. One possible reason for the permeability increase may be the release of proteases from degenerate granulocytes, as this has been claimed to cause \( \text{K}^{+} \) leakage from red cells and hemolysis.\(^{32} \) Another possibility may relate to membrane lipid changes at 4°C, since Haradin et al.\(^{6} \) have demonstrated a progressive loss of both phospholipid and cholesterol from the membrane during cold storage. Still another possibility is the increasing attachment of the third component of complement (fragment C3c) to the red cell, which has been detected on cold-stored red cells by an autoanalyzer technique that added polyvinylpyrrolidone to enhance sensitivity.\(^{12} \) However, C3 attachment must be slight, since we failed to detect C3 on stored cells using a manual (tube) Coombs technique. Fresh red cells coated with C3 and C4 by prior exposure to cold agglutinin did show an increment in \( \text{Ca}^{2+} \) permeability that ranged from 20% to 45%. However, massive complement coating is required for this effect and the many-fold increase in \( \text{Ca}^{2+} \) permeability of stored cells is unlikely to result from a complement deposition which is below the limit of detection with a manual Coomb’s technique. Can our results be explained by the calcium paradox phenomenon? Exposure of heart muscle to \( \text{Ca}^{2+} \)-free media increases its resting \( \text{Ca}^{2+} \) permeability many-fold,\(^{23} \) and this phenomenon superficially resembles the development of \( \text{Ca}^{2+} \) leakiness in erythrocytes cold-stored in citrate anticoagulants (ionized \( \text{Ca}^{2+} \) ca. 60 \( \mu M \)). However, erythrocytes cold-stored in heparin anticoagulant with ionized \( \text{Ca}^{2+} \geq 1 \) \( \mu M \) showed an identical increase in \( \text{Ca}^{2+} \) permeability to cells in CPD so that low ambient \( \text{Ca}^{2+} \) during cold storage cannot explain the permeability increase. In contrast, the ambient pH seems to be an important factor, since \( { }^{45}\text{Ca}^{2+} \) uptake rose more promptly and to a higher value in ACD (pH 7.0) than in CPD (pH 7.2–7.3) anticoagulants.

Direct measurements of the \( \text{Ca}^{2+} \) concentration of stored erythrocytes sampled directly from the bag failed to show a significant difference from fresh erythrocytes, which is in agreement with the results of Haradin et al.\(^{6} \) The value observed for total red cell \( \text{Ca}^{2+} \) concentration (4.3 ± 1.9 nmole/ml cells) is similar to that obtained previously in our laboratory using a dry ashing technique (5.9 ± 1.8 nmole/ml cells)\(^{3} \) and to the data of Lew,\(^{24} \) although it is lower than values obtained by others.\(^{6,25,26} \) Meticulous attention was paid to the removal of Buffy coat, since platelets contain large amounts of calcium and all the washing media were passed through a Chelex-100 column to remove the 1–3 \( \mu M \) \( \text{Ca}^{2+} \) contaminant invariably present in saline solutions. The normal \( \text{Ca}^{2+} \) content of cold-stored erythrocytes is puzzling, although the addition of blood to citrate-based anticoagulants lowers the ionized (free) \( \text{Ca}^{2+} \) concentration of plasma to less than 100 \( \mu M \), which therefore reduces the concentration gradient for \( \text{Ca}^{2+} \) movement into the red cell. When erythrocytes were incubated in vitro under conditions that simulate a plasma environment (i.e., 1.5 mM ionized \( \text{Ca}^{2+} \) with glucose present), the stored cells showed a net gain of \( \text{Ca}^{2+} \), which was significantly greater than for fresh erythrocytes. It is not clear whether a similar net increase in red cell calcium occurs when blood is transfused to a recipient. Storage “lesions” may be reversed, since the low red cell ATP and 2,3-DPG are rapidly repleted after transfusion of the cold-stored cells to a recipient.\(^{27,28} \) Thus, any increase in cell \( \text{Ca}^{2+} \) permeability is likely to be transient since the majority of transfused red cells survive normally.

Numerous deleterious effects of increased intracellular \( \text{Ca}^{2+} \) have been described and an influx of \( \text{Ca}^{2+} \) has been proposed as a final common pathway for cell death induced by many toxins.\(^{29} \) It has been suggested that the greater \( \text{Ca}^{2+} \) leakiness of stored erythrocytes may produce a metabolic drain on ATP due to its consumption by the calcium pump.\(^{30,31} \) However, Szasz et al.\(^{14} \) have calculated that not more than 5% of the normal ATP production is needed for the extra demands of \( \text{Ca}^{2+} \) pumping on reincubation of stored erythrocytes. Intracellular \( \text{Ca}^{2+} \) may also induce an

\(^{\dagger} \) ACD-plasma ionized \( \text{Ca}^{2+} \) is 62 \( \mu M \) calculated from a stability constant for Ca-citrate of \( 1.26 \times 10^{5} \text{M}^{-1} \), final citrate concentration 19.0 \( \text{mM} \), and calcium not bound to albumin of 1.5 \( \text{mM} \).
efflux of K⁺ via the Ca²⁺-stimulated K⁺ channel present in human erythrocytes.¹³,33 However, in ATP-replete erythrocytes incubated with low concentrations of ionophore A23187, this K⁺ channel has a high apparent Kₘ for internal Ca²⁺ between 0.33 and 1.5 mM,³⁴ which is well above the levels of cell Ca²⁺-attained on reincubation of stored erythrocytes. Our study demonstrates that no loss of cell K⁺ occurs on reincubation of cold-stored erythrocytes in saline media containing glucose and Ca²⁺, although the cell Ca²⁺ content more than doubled after 1-hr incubation. Thus, Ca²⁺-stimulated K⁺ efflux with net loss of ions and water may not be significant to transfused cells except when the red cell becomes depleted of ATP. Finally, red cells incubated with Ca²⁺ plus ionophore A23187 accumulate 1,2-diacylglycerol, which is thought to result from the action of a Ca²⁺-stimulated phospholipase on tri- and diphosphoinositides.³⁴ Diacylglycerol may be responsible for the echinocytic shape change induced by Ca²⁺ in erythrocytes from a variety of species,³⁵ and indeed, its accumulation in stored erythrocytes³⁶ has been suggested as a factor in this morphological change. Alternately, intracellular Ca²⁺ may produce the echinocytic shape by reversible modification of the spectrin-actin cytoskeleton, which is physically linked to the membrane via the transmembrane protein, glycoporphin.³⁷ Whether an increase in cell Ca²⁺ content may contribute to the destruction of nonviable cells within the first 24 hr after transfusion merits study. The present data show that a selective increase in Ca²⁺ permeability is induced by cold storage of human erythrocytes, and this change precedes any fall in cell ATP concentration.

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