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The Homogeneous Effect of Calcium Ionophore A23187 on Potassium Loss in Human Foetal Red Cell Populations

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Summary

A "pulse like" increase of cytoplasmic calcium concentration, which is proportional to ionophore concentration, is induced in red cells by exposure to A_{23187}. Different Ca^{2+} levels are attained depending on cellular calcium buffering power and/or primary active calcium transport activation. We examined the effect of A_{23187} concentration on potassium loss in neonatal (nRC) as well as in adult red cells (aRC). The increase in ionophore concentration produced an "all- or -none" recruitment in adult cells and a "gradual" one in neonatal red cells. The "gradual" response observed in nRC would suggest that the "all or none" character of the response is not present in red cells during the foetal stages of haematopoiesis.

Key words

Foetal red cells – Calcium Ionophore – Potassium loss

Introduction

Red cells from the late foetal period of development are final products of the hepato-splenic haematopoietic (red cell lineage) stage (Tavassoli 1991). They are formed by cellular cohorts with a compact life span distribution (Pearson 1967, Matovik *et al.* 1986) that differ from adult red cells in a variety of characteristics, both morphological and functional (Schekman and Singer 1976, Linderkamp *et al.* 1983, Matoth *et al.* 1971, Serrani *et al.* 1991, Serrani and Corchs 1987).

We analyzed the effect of a calcium ionophore (A_{23187}) on cellular potassium loss in adult and neonatal red cells. A_{23187} is a compound widely used in the study of calcium signalling phenomena (Pfeiffer *et al.* 1978, Lew and Garcia-Sancho 1985). We observed that the number of cellular cohorts involved in potassium leakage to increasing A_{23187} concentration, progressed in a continuous or "gradual" fashion in neonatal red cells as opposed to the heterogeneous or "all-or-none" effect observed in adult red cells. This different behaviour could be the result of the induction of potassium release from specific subpopulations of adult red cells by the ionophore (recruited in an "all-or-none" manner from the total cellular stores and in proportion to the cell calcium concentration) and inv olvement of all cellular nRC subpopulations in potassium release and proportionally to calcium levels.

Materials and Methods

Human blood samples from normal adults and from umbilical cords of normally delivered infants were used within five hours after extraction. Each sample was centrifuged to remove the plasma; the cell pellet was resuspended in a saline-buffered medium and centrifuged again. This procedure was repeated three times to guarantee that the erythrocytes were free of plasma. The cells were then suspended at a 2–3 % haematocrit in a medium with the following mM composition: Na 120; K 1; Ca 1; NO_3 122; Tris-MOPS 10 (pH 7.4, at 37 °C); glucose 10.

The cell suspension was incubated in a thermostatized, lateral shaking water bath, with variable concentrations of the ionophore A_{23187}. Aliquots were withdrawn at different incubations times and centrifuged in a Beckman microfuge; the cell pellet

and supernatant were collected for further estimation. The fractional haemolysis of the suspensions after 15 min incubation amounted to 0.02.

Cells were lysed in redistilled water to determine intracellular sodium and potassium; the latter was also measured in the supernatants (extracellular potassium). Cation concentrations were measured by atomic absorption spectrophotometry in a Perkin Elmer spectrophotometer model 2380. The osmolality and pH of the incubation media were determined with an Advanced Instruments Osmometer and a Beckman Expandomatic pHmeter.

The relative cellular volume (rcv) was estimated as described by Dunham and Ellory (1981). Briefly, the haemoglobin concentration/haematocrit ratio (Hb/Hct) of cellular suspensions with a Hct value around 50 % was obtained before and after incubation; the quotient of these ratios estimates rcv. The cellular density of incubated suspensions was obtained as reported elsewhere (Lauf 1983).

Cellular ATP was determined by the NADH-NAD conversion method using the enzyme preparation from Sigma Chemical Co. (Saint Louis, MO, USA). This was expressed in μmoles/g Hb.

The extracellular potassium concentration was analyzed as a function of time after addition of the ionophore to the cell suspensions. The data were fitted with the following analytical expression, corresponding to a bicompartmental system (Sten-Knudsen 1979): K_o = limit [1 - exp (-kt)]

The parameter k of the system expresses the time course of the transformation from a "fast" to a "slow" phase of K^+ increments in the medium. This analysis was carried out using an ENZFITTER program. However, if the correlation coefficient was greater than 0.95, the experimental points corresponding to the initial (fast) phase were studied by means of linear regression analysis.

The results were expressed as means ± S.E.M. Due to the normal variation among donors in the absolute rate of cellular K^+ transport, the data were not pooled in some experiments (representative studies are shown in the figures). In each case, similar results were obtained with cells from three or more different donors.

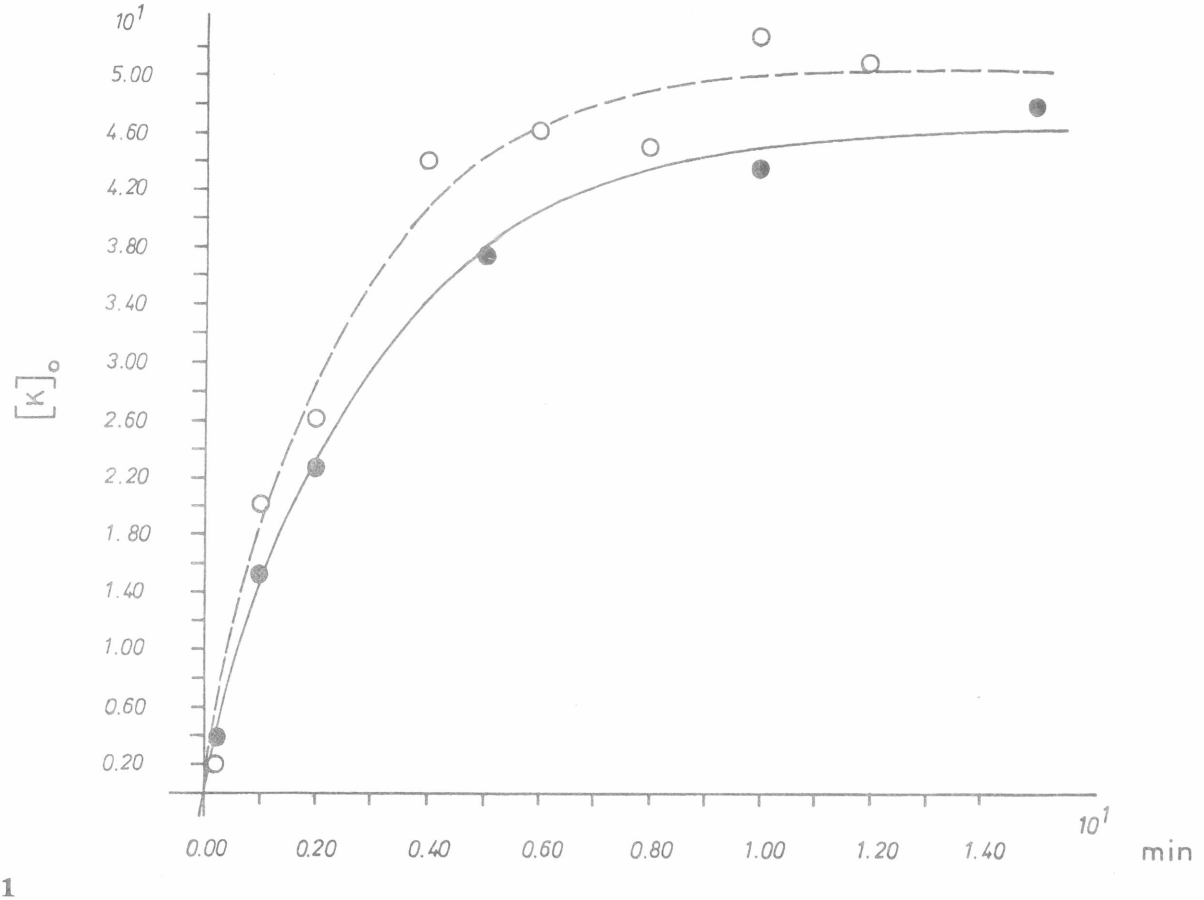

Fig. 1

Changes of extracellular potassium concentration (ppm) as a function of time, after adding 1 μM A_{23187} to a suspension of adult (aRC, open circles) or neonatal (nRC, full circles) red cells incubated as described in Materials and Methods. Data from one representative experiment are shown. A first order rate equation fitted both sets of data, with parameters k equal to 0.43 ± 0.05 (aRC) and 0.35 ± 0.02 (nRC), and limits equal to 50.15 ± 1.63 (aRC) and 43.35 ± 1.10 (nRC) (data from seven different individuals).

Results

Cellular volume. Both neonatal and adult red cells were reduced in volume because of the depletion of cellular osmolytes induced by the ionophore. This cell shrinkage, estimated by rcv after 15 min of incubation, was significantly larger in neonatal red cells (0.70±0.04, n=7) than in adult red cells (0.85±0.05, n=7) ($p<0.05$). The rcv obtained in the absence of ionophore A_{23187} in nRC and aRC amounted to 1.04±0.02 (n=7) and 1.05±0.01 (n=7), respectively.

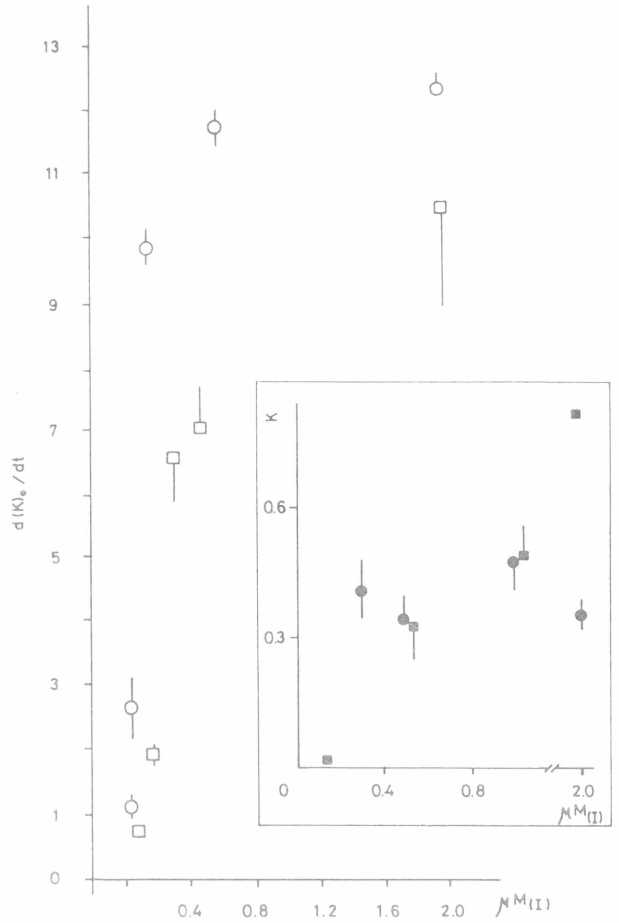

Fig. 2
Initial rate of potassium loss as a function of the ionophore concentration, in red cell suspensions incubated with A_{23187}; aRC (open squares), nRC (open circles). Insert: values of the *k* parameter (estimated for different A_{23187} concentrations with the first order rate equation as shown in Fig. 1) are presented as a function of the ionophore concentration for aRC (full squares) and nRC (full circles). Vertical bars: S.E.M.

Cellular contents of potassium and sodium. After 15 min of incubation with A_{23187}, the two cell types decreased their potassium content to 50 % or less (55.86±2.95 (n=13) and 51.84±4.00 (n=17) for nRC and aRC, respectively) of the corresponding control values (119.9±10.00 (13) and 103.68±4.68 (17) in nRC and aRC, respectively).

Control values of the sodium content were 16.86±1.34 (nRC, n=10) and 10.76±1.25 mmol/l (aRC, n=11). The treatment did not significantly modify cellular sodium (nRC 18.70±1.00, n=10; aRC 11.04±1.20, n=11).

Time course of extracellular potassium concentration changes after ionophore addition. The data of the whole time course were fitted by a first order rate equation corresponding to a bicompartmental system. The analytical expression employed was linearized by a logarithmic transformation. Fig. 1 shows the data and the curve fitting parameters for an ionophore concentration (in the extracellular phase) equal to 1 μM.

The initial phase of changes in extracellular potassium concentration followed a linear pattern during the first 2–3 min after addition of the ionophore. Therefore, regression analysis could be used to study this period. Only those sets of experimental points with regression coefficient greater than 0.95 were selected for analysis. The initial rate of cellular potassium loss was estimated with a slope of the analytical expression (Fig. 2).

Standardization of *k* and dK^+/dt parameters. Fig. 3 demonstrates the effect of A_{23187} on time dependence of extracellular potassium concentration, at three extracellular calcium concentrations, varying in three orders of magnitude. *k* parameters were referred to the corresponding limit parameters. dK^+/dt parameters were obtained from the first points of the extracellular time-dependent K^+ concentration and they were referred to the corresponding limit parameters.

Cellular ATP. At the end of the incubation period, the ATP content in nRC was 4.40±0.59 (n=5) μmoles/g Hb. This result did not differ significantly from that reported for unincubated aRC (3.96±0.2, n=7) and nRC (3.78±0.1, n=7) (Corchs *et al.* 1993, Sarkady and Tosteson 1979).

Discussion

Ionophores, though having carrier-like properties, produce cationic equilibrium distribution through membranes (mitochondrial, thylakoid, cell surface). One of these organic compounds, A_{23187}, is widely used to study calcium signalling phenomena in eukaryotic cells (Pfeiffer *et al.* 1978, Pyant and Brierley 1982).

Partitioning of this compound in cell surface membranes induces a homogeneous cytoplasmic calcium increase in every cell of a mixed subpopulation suspension, proportional to the ionophore concentration (Lew and Ferreira 1976), and related to $Ca^{2+}{}_o/2H^+{}_i$ exchange. The final level of Ca^{2+}

depends on intervening factors such as "calcium pump" activity and cytoplasmic calcium buffering. Increase in intracellular calcium induces a potassium leak in red cells (Gardos' effect) (Toro and Stefani 1991, McManus 1991, Scharff and Foder 1986, Leinders *et al.* 1992a,b).

Our analysis of the effect of A_{23187} on potassium loss in both nRC and aRC was done on the whole time course of potassium leakage as well as on the initial phase of the loss. The range of ionophore concentrations tested was selected to avoid undesirable cellular effects of the compound (White 1974, Taylor *et al.* 1977, Klausner *et al.* 1979, Engstrom *et al.* 1993). The results suggest the presence of calcium-activated potassium channels of the "mini" type in aRC (Leinders *et al.* 1992a,b, Canessa 1991). nRc individual channel conductance was in the order of pS units, but the cellular density needed to achieve this magnitude was double compared to aRC (data not shown).

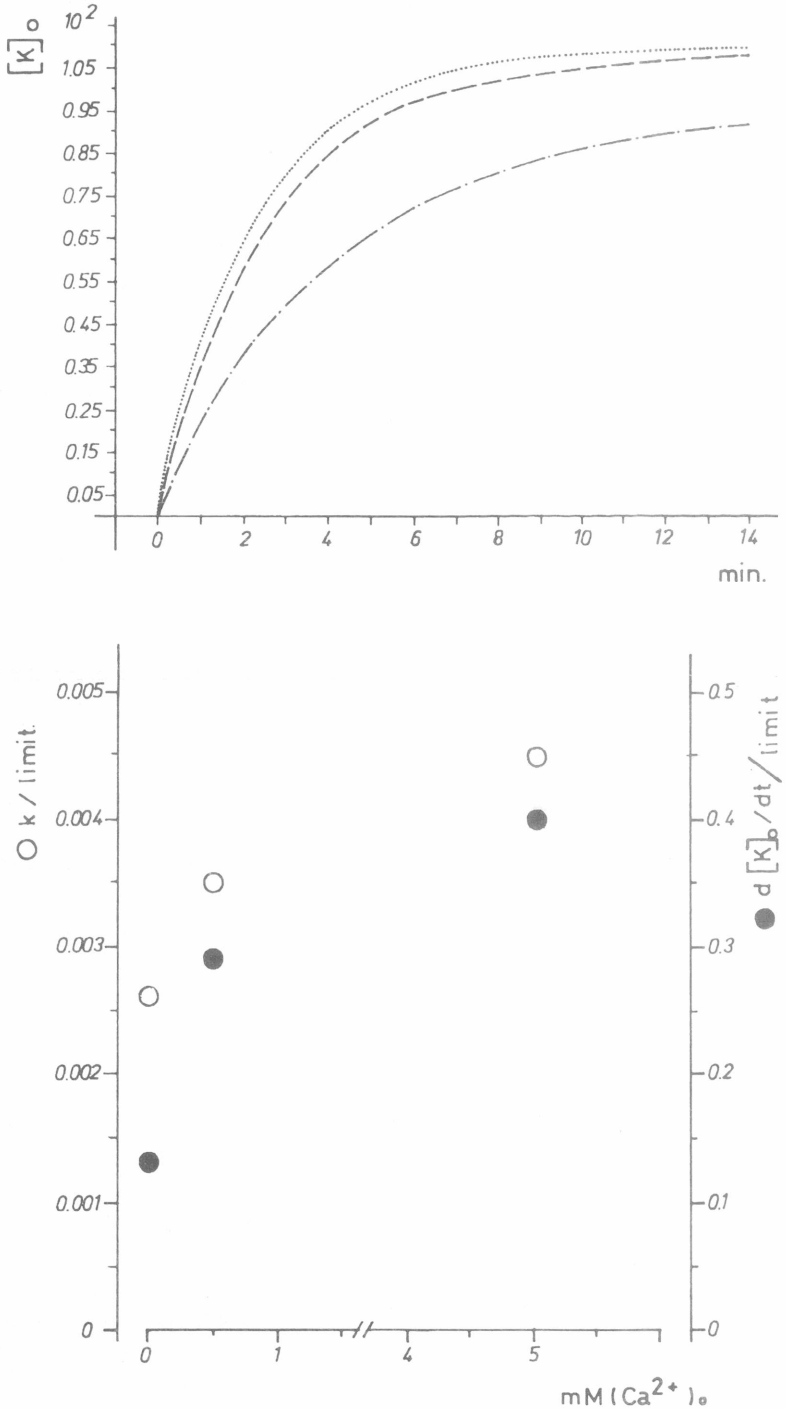

Fig. 3
Relationship in neonatal red cells between extracellular calcium concentration and the parameters *k* (open circles) and $d[K]^+/dt$ (full circles) standardized for the corresponding limit parameters. nRC were incubated as reported in Material and Methods, in the presence of 1 μM A_{23187} and the calcium concentrations given in the abscissa. Upper panel: the first order rate equation fitting the data is shown for the following (mM) Ca^{2+} concentrations: 0.005 (points, lines), 0.5 (lines) and 5 (points). In order of increasing Ca^{2+} concentrations the data for aRC corresponding to the k/limit ratio were: 0.006 ± 0.002; 0.007 ± 0.001 and 0.006 ± 0.001 respectively. In these cells, the values for the $d[K^+]/dt$/limit ratio for the corresponding Ca^{2+} concentrations were 0.15 ± 0.04; 0.15 ± 0.01 and 0.10 ± 0.01, respectively (data from five different individuals.

The initial rate of potassium leakage observed in both cell types (Fig. 2), when the A_{23187} concentration increased, could be due to the recruitment of different cell subpopulations rather than individual channels of selected subpopulations. This hypothesis is derived from the range of extracellular calcium and ionophore concentrations tested as well as from the threshold of channel activation by calcium.

The analysis of the *k* parameter as a function of A_{23187} showed independence for nRC and a positive linear association for aRC. This disparate behaviour could be explained on the basis of a different cell involvement in potassium loss – it is homogeneous or "gradual" for nRC, while it is heterogeneous or "discontinuous" for aRC. This kind of response has been described for the adult cells (Knauf *et al.* 1975, Colombe and Macey 1974, Riordan and Passon 1971, Yingst and Hoffman 1984). The finding that the initial rate/limit and the k/limit ratios were associated in nRC also supports our interpretation of the data (Fig. 3). Differences in structural and functional parameters have been shown to occur in red cells in the course of (i) the maturation process, (ii) the aging of mature cells and (iii) the developmental stages of the individual (foetal-adult-senescent) (Clark 1988, Aiken *et al.* 1992, Gaczynska 1989, Jain 1988, Winterbourn and Batt 1970, Westerman *et al.* 1963, Shiga *et al.* 1979, Sutera *et al.* 1985).

The results presented in this paper would serve as another instance of the differences arising under (iii).

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References

AIKEN N.R., SATTERLEE J.D., GALEY W.R.: Measurement of intracellular Ca^{2+} in young and old human erythrocytes using ^{19}F-NMR spectroscopy. *Biochim. Biophys. Acta* **11:** 155–160, 1992.

CANESSA M.: Red cell volume-related transport systems in hemoglobinopathies. *Haematology/Oncology Clinics of North America* **5:** 495–516, 1991.

CLARK M.R.: Senescence of red blood cell: Progress and problems. *Physiol. Rev.* **68:** 503–554, 1988.

COLOMBE BW., MACEY R.I.: Effects of calcium on potassium and water transport in human erythrocyte ghosts. *Biochim. Biophys. Acta* **363:** 226–239, 1974.

CORCHS J.L., CORCHS M.J., SERRANI R.E.: Neonatal red blood cell lysis induced by hypertonic low ionic strength media. *Arch. Int. Physiol. Biochim. Biophys.* **101:** 249–252, 1993.

DUNHAM P.B., ELLORY J.C.: Passive transport in low potassium sheep red cells: dependence upon cell volume and chloride. *J. Physiol. Lond.* **318:** 511–530, 1981.

ENGSTROM I., WALDENSTROM A., RONQUIST G.: Ionophore A_{23187} reduces energy charge by enhaced ion pumping in suspended human erythrocytes. *Scand. J. Clin. Lab. Invest.* **53:** 239–246, 1993.

GACZYNSKA M.: Changes in the proteolytic activity of human erythrocyte membrane during red cell aging. *Biochim. Biophys. Acta* **981:** 173–177, 1989.

JAIN S.K.: Evidences for membrane lipid peroxidation during in vivo aging of human erythrocytes. *Biochim. Biophys. Acta* **937:** 205–210, 1988.

KLAUSNER R.D., FISHMAN M.C., KARNOVSKY M.J.: Ionophore A_{23187} disrupts structure by modifying protein-lipid interactions. *Nature* **281:** 82–83, 1979.

KNAUF P.A., RIORDAN J.R., SCHUHMANN B., WOOD-GUTH I., PASSOW H.: Calcium-potassium-stimulated net potassium efflux from human erythrocyte ghosts. *J. Membr. Biol.* **25:** 1–22, 1975.

LAUF P.K.: Thiol-dependent passive K/Cl transport in sheep red cells. I. Dependence on chloride and external K^+ [Rb^+] ions. *J. Membr. Biol.* **73:** 237–246, 1983.

LEINDERS T., VAN KLEEF R.G.D.M., VIJVERBERG H.P.M.: Distinct metal ion binding sites on Ca^{2+}-activated K^+ channels in inside-out patches of human erythrocytes. *Biochim. Biophys. Acta* **1112:** 75–82, 1992a.

LEINDERS T., VAN KLEEF, R.G.D.M., VIJVERBERG H.P.M.: Single Ca^{2+}-activated K^+ channels in human erythrocytes: Ca^{2+} dependence of opening frequency but not open lifetimes. *Biochim. Biophys. Acta* **1112:** 67–74, 1992b.

LEW V.L., FERREIRA H.G.: Variable Ca sensitivity of a K-selective channel in intact red-cell membranes. *Nature* **263:** 336–338, 1976.

LEW V.L., GARCIA-SANCHO J.: Use of the ionophore A_{23187} to measure and control cytoplasmic Ca^{2+} levels in intact red cells. *Cell Calcium* **6:** 15–23, 1985.

LINDERKAMP O., WU P.Y., MEISELMAN H.J.: Geometry of neonatal and adult red blood cells. *Pediatr. Res.* **17:** 250–253, 1983.

MATOVIK L.M., CHIU D., LUBIN B., MENTZER W., LANE P.A., MOHANDAS N., SCHRIER S.: The aging process of human neonatal erythrocytes. *Pediatr. Res.* **20:** 1091–1096, 1986.

MATOTH Y., ZAIZOV R., VARSANO I.: Postnatal changes in some red parameters. *Acta Paediat. Scand.* **60:** 317–323, 1971.

MCMANUS O.B.: Calcium-activated potassium channels: regulation by calcium. *J. Bioenerg. Biomembr.* **23:** 537–560, 1991.

PEARSON H.A.: Life-span of the foetal red blood cell. *J. Pediatr.* **70:** 166–171, 1967.

PFEIFFER D.R., TAYLOR R.W., LARDY H.A.: Ionophore A_{23187}: cation binding and transport properties. *Ann. N.Y. Acad. Sci.* **307:** 402–423, 1978.

PYANT K.S., BRIERLEY G.P.: Monovalent cation conductance in liposomes induced by ionophore A_{23187}. *Experientia* **38:** 1202–1204, 1982.

RIORDAN J.R., PASSAW H.: Effects of calcium and lead on potassium permeability of human erythrocyte ghosts. *Biochim. Biophys. Acta* **249:** 601–605, 1971.

SARKADY B., TOSTESON D.C.: Transport Across Single Biological Membranes. In: *Membrane Transport in Biology.* GIEBISCH G., TOSTESON D.C., USSING H.H. (eds), Springer, Berlin, 1979. pp. 117–160.

SERRANI R.E., CORCHS J.L.: Furosemide-sensitive sodium and potassium fluxes in human neonatal erythrocytes. *Arch. Int. Physiol. Biochem.* **95:** 341–346, 1987.

SERRANI R.E., GIOIA I.A., CORCHS J.L.: Human neonatal red cells. Regulatory volume response under anisotonic conditions. *Arch. Int. Physiol. Biochim. Biophys.* **99:** 473–477, 1991.

SCHARFF O., FODER B.: Delayed activation of calcium pump during transient increases in cellular Ca^{2+} concentration and K^+ conductance in hyperpolarizing human red cells. *Biochim. Biophys. Acta* **581:** 471–479, 1986.

SCHEKMAN R., SINGER S.J.: Clustering and endocytosis of membrane receptors can be induced in mature erythrocytes of neonatal but not adult humans. *Proc. Natl. Acad. Sci. USA.* **73:** 4075–4079, 1976.

SHIGA T., MAEDA N., SUDA T., KOW K., SEKIYA M.: The decreased membrane fluidity of in vivo aged human erythrocytes. A spin label study. *Biochim. Biophys. Acta* **553:** 84–95, 1979.

STEN-KNUDSEN O.: Passive Transport In: *Membrane transport in biology.* GIEBISCH G., TOSTESON D.C., USSING H.H. (eds), Springer, Berlin, 1979, pp. 5–13.

SUTERA S.P., GARDNER R.A., BOYLAN C.W., CARROLL G.L., CHANG K.C., MARVEL J.S., KILO B., SON J.R.W.: Age-related changes in deformability of human erythrocytes. *Blood* **65:** 275–282, 1985.

TAYLOR D., BARER R., HOCHSTEIN P.: The effect of calcium ionophore A_{23187} on the ATP level of human erythrocytes. *Biochem. Biophys. Res. Commun.* **76:** 205–211, 1977.

TAVASSOLI M.: Embryonic and foetal hemopoiesis: An overview. *Blood Cells* **1:** 269–281, 1991.

TORO L., STEFANI E.: Calcium-activated potassium channels: metabolism regulation. *J. Bioenerg. Biomembr.* **23:** 561–576, 1991.

WESTERMAN P., PIERCE E., JENSEN W.N.: Erythrocyte lipids: a comparison of normal young and normal old populations. *J. Lab. Clin. Med.* **62:** 394–400, 1963.

WINTERBOURN C.C., BATT R.D.: Lipid composition of human red cells of different ages. *Biochim. Biophys. Acta* **202:** 1–8, 1970.

WHITE J.G.: Effects of an ionophore, A_{23187}, on the surface morphology of normal erythrocytes. *Am. J. Pathol.* **77:** 507–514, 1974.

YINGST D.R., HOFFMAN J.F.: Ca-induced K transport in human red blood cell ghosts containing arsenazo III. Transmembrane interactions of Na, K, and Ca and the relationship to the functioning Na-K pump. *J. Gen. Physiol.* **83:** 19–41, 1984.

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