J. Membrane Biol. 26, 357–370 (1976)

The Use of Ionophores of Rapid Loading of Human Red Cells with Radioactive Cations for Cation-Pump Studies

B. Sarkadi, Ilma Szász, and G. Gárdos

Department of Cell Metabolism, National Institute of Haematology and Blood Transfusion, Budapest, Hungary

Received 8 July 1975; revised 10 November 1975

Summary. Techniques are described for the rapid loading of intact human red cells with radioactive isotopes of alkali cations or Ca^{2+} by using ionophorous compounds (nigericin, gramicidin D and A 23187). Loading was rapid and efficient if the membrane potential of the cells was rendered more negative inside. After cation loading the ionophores could be bound to albumin and removed by repeated washings. The ATP and 2,3-DPG contents of the cells were practically unaltered by this treatment. Passive membrane permeability to Na^+ and Ca^{2+} returned to normal. Loaded erythrocytes pumped out Na^+ in a ouabain-sensitive and Ca^{2+} in a lanthanum-sensitive way. Ca^{2+}-loaded red cells were microspherocytes and exhibited a rapid K^+-efflux. Parallel with the extrusion of Ca^{2+} cells regained their biconcave shape and normal passive permeability to K^+.

In order to investigate alkali cation and Ca^{2+} efflux from red cells at stationary conditions, radioactive isotopes are to be introduced into the cells. Owing to the low cation permeability of the red cell membrane, incubation for several hours at 37 °C or for several days at 4 °C is needed to achieve even a relatively poor loading with alkali cations (Harris & Prankerd, 1953; Glynn, 1956; Garrahan & Glynn, 1967; Lubowitz & Whittam, 1969). The hazard run in such a procedure is that the metabolic state and membrane parameters of the red cells may change. Moreover, Ca^{2+}-ions practically cannot be introduced by these simple means (Schatzmann & Vincenzi, 1969). The special treatments recommended, like the "lactose procedure" (Whittam & Ager, 1965) for alkali cation loading, the PCMBS (Garrahan & Rega, 1967; Schatzmann, 1973) and the trinitrocresol treatment (Gunn & Tosteson, 1971; Dunn, 1974) for alkali cation- and Ca^{2+}-loading also share the above shortcomings. The same holds if nystatin, a nonselective polyene ionophorous antibiotic, is used according to the method of Cass and Dalmark

(1973). In addition, the elimination of the ionophore in this case is not satisfactorily solved.

Cation species can also be introduced into the cells by reversible haemolysis and the resealed ghosts are then used in transport experiments. Excellent reviews are available surveying the difficulties of the ghost-technique (Bodemann & Passow, 1972; Schwoch & Passow, 1973). Particular care is to be exercised obtaining appropriate Ca^{2+}-loaded ghosts (Schatzmann, 1973), as their Ca^{2+}-pump function may easily get impaired (Szász, Gárdos & Árky, 1971).

In the present work our aim was to find relatively mild techniques for introducing labelled cations into intact red cells. Our approach was based on the experimental results of Ekman, Manninen and Salminen (1969) as interpreted by Glynn and Warner (1972). According to them the induction of K^+ efflux into media containing nonpenetrating cations generates a potential which serves as an additional driving force for the influx of labelled cations.

We attempted to achieve rapid cation loading of red cells by meeting the following criteria: (1) The cell membrane be highly permeable to K^+. (2) The cell membrane be highly permeable to the labelled cation to be introduced. (3) The use of an incubation medium whose main cation practically cannot penetrate the cell membrane.

Criteria 1 and 2 were fulfilled by the application of ionophores as described below. Due to the change in the membrane potential generated by the rapid K^+ efflux an efficient loading with radioactive cation isotopes was obtained within a short time. The loading procedure could be terminated and the original permeability of the membrane restored by the elimination of the ionophores through binding to albumin.

Materials and Methods

Chemicals: ouabain (Fluka), oligomycin (Serva), chlorobutanol (Richter), $LaCl_3$ (Reanal) and gramicidin D (Calbiochem) were of reagent grade. Propranolol, A 23187, and nigericin were kindly provided by V. Manninen, E. Carafoli and I. Horváth, respectively.

Washed red cells from freshly drawn, heparinized human blood were used.

Na^+ and K^+ were determined by flame-photometry with an EEL photometer. Intracellular Na^+ and K^+ were measured in the TCA extract of cells washed three times with cold isosmotic cholinechloride.

^{42}K (specific activity = 0.159 Ci/g), ^{86}Rb (0.23 Ci/g), ^{22}Na (188 Ci/g) and ^{125}I (1 μCi/mg prot.) activities were counted in a Beckman Biogamma, whereas ^{45}Ca (2.15 Ci/g) was counted in an Intertechnique SL-30 liquid scintillation spectrometer. For ^{45}Ca countings made with 0.2 ml TCA extract in 10 ml scintillation liquid (Liquid Scintillator-Nuclear

E.L.—diluted with toluene and mixed with an equal volume of absolute ethanol) no correction for quenching was needed.

Haemoglobin was measured by the cyanomethaemoglobin method. The haematocrit value was assessed by the microhaematocrit tube technique or by ^{125}I dilution.

ATP was determined by the aid of Boehringer UV-test; 2,3-DPG by means of the Calbiochem 2,3-DPG kit.

Morphology of the formaldehyde-fixed red cells was checked under the phase-contrast microscope and characterized by the morphological index, I_m (Gárdos, Szász & Árky, 1966), which equals 100 for homogeneous biconcave disk and 0 for homogeneous microspherocyte populations.

Tracer influx was studied in suspensions of 20–30% haematocrit. The ingredients were mixed at 0 °C, then the suspension was transferred into a water bath adjusted to 37 °C and shaken vigorously. Red cells of 0.5 ml samples were separated by rapid centrifugation (1 min, $10,000 \times g$) through an ice-cold, 7 ml sucrose cushion (0.7 M sucrose in 0.16 M NaCl). The red cell pellet was rinsed thoroughly with 0.16 M NaCl and haemolysed with 1.0 ml of 0.1% saponin.

Tracer efflux from the loaded cells was followed in a suspension of 3–5% haematocrit. Samples were centrifuged at $10,000 \times g$ for 1 min, and aliquots of the supernatants were counted.

Loading with alkali cation isotopes was carried out in the following steps:

(1) Red cells from 20 ml freshly drawn blood were washed 3 times with isosmotic cholinechloride solution. The packed cells were kept in an ice-cold water-bath.

(2) The following medium was prepared and cooled to 0 °C: 2.5 ml of 0.16 M cholinechloride, pH 7.4 (buffered with 0.15 mM tris-HCl) +1.0 ml of 50 μM nigericin or 2 μM gramicidin D+0.6 ml of $^{22}NaCl$, ^{42}KCl or $^{86}RbCl$. Stock solutions of antibiotics were prepared in absolute ethanol. Before use a tenfold dilution was made of them in isosmotic cholinechloride solution.

(3) The packed red cells (6.0 ml) were mixed with the above medium in a small Erlenmeyer flask. The suspension was incubated at 37 °C for 1 min under vigorous shaking.

(4) The suspension was poured into 500 ml of ice-cold isosmotic cholinechloride solution containing 0.5% human albumin (pH 7.4) and was washed with the same volume of cold choline-albumin solution three times within 15 min.

The ^{45}Ca-loading procedure consisted of the following steps:

(1) Red cells from 20 ml freshly drawn blood were washed three times with isosmotic NaCl solution. The packed red cells were kept in an ice-cold water bath.

(2) The following incubation medium was prepared and cooled to 0 °C: 3.3 ml of 0.16 M NaCl solution, pH 8.0 (buffered with 0.15 mM Tris-HCl) +0.2 ml of 10^{-4} M A-23187 in absolute ethanol +0.5 ml of 0.16 M NaCl replaced in part by 0.11 M $CaCl_2$ and by the $^{45}CaCl_2$ stock solution to obtain final Ca^{2+} concentrations of 0.01–10.0 mM.

(3) The packed red cells (6.0 ml) were mixed with the above medium in a small Erlenmeyer flask. The suspension was incubated at 37 °C for 2 min under vigorous shaking.

(4) The suspension was poured into 500 ml of ice-cold 0.16 M KCl solution containing 0.5% human albumin (pH 7.4) and was washed with the same volume of cold KCl-albumin solution three times within 15 min.

Results

Rapid Loading Techniques

Red cells incubated with nigericin or gramicidin D in isosmotic cholinechloride medium lose their K^+ and Na^+ ions rapidly (Fig. 1*a*). Con-

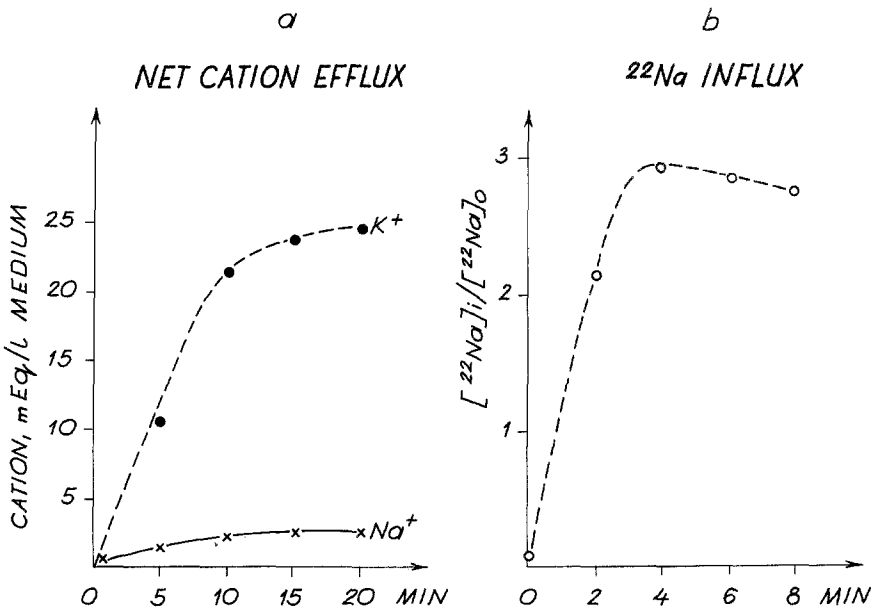


Fig. 1. Effect of 5 μM nigericin on net alkali cation loss (*a*) and ^{22}Na uptake (*b*) of erythrocytes. Note the difference between the time-scales of *a* and *b*. Incubation medium: 0.16 M choline chloride (pH 7.4). $t = 37$ °C; haematocrit = 30%; specific activity of $^{22}NaCl$ = 6.0 mCi/mmole

comitantly, a rapid tracer K^+ and/or Na^+ uptake can be observed. In the first minutes of incubation the uptake of labelled cations results in an isotope concentration several times as high inside the cells as in the medium (Fig. 1 *b*). During the further net cation loss the labelled cations tend to reach their concentration-equilibria.

Compound A 23187 is a divalent cation-ionophore and alkali cations are not transported by it (Reed & Lardy, 1972). However, in the presence of Ca^{2+} ions A 23187 evokes a rapid K^+ transport in the human erythrocytes without any significant effect on the Na^+ permeability (Reed, 1973; Gárdos, Sarkadi & Szász, 1975). Due to the rapid K^+ efflux, in NaCl medium the ionophore-mediated Ca^{2+} influx is significantly faster than in KCl medium, where net K^+ efflux cannot occur. If chlorobutanol, an inhibitor of rapid K^+ efflux, is added to the NaCl medium, Ca^{2+} influx is hindered to the same extent (Fig. 2).

According to the above results, by terminating the high cation permeability of the red cell membrane in the first minutes cells can be obtained in which the labelled cation concentration may exceed that of the medium, while the intracellular cation composition remains practically unaltered.

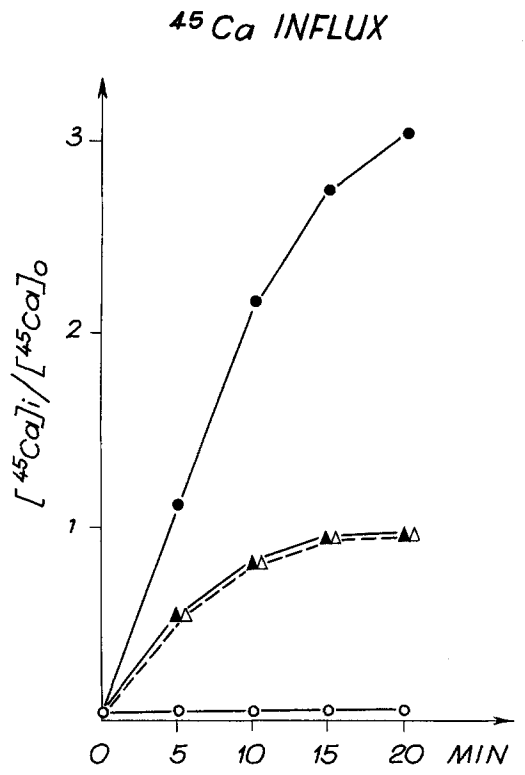


Fig. 2. ^{45}Ca uptake of human erythrocytes in the presence of A 23187. o—o 0.16 M NaCl; ●—● 0.16 M NaCl+5 μM A 23187; ▲—▲ 0.16 M NaCl+5 μM A 23187+3 mM chlorobutanol; △—△ 0.16 M KCl+5 μM A 23187; $t=37$ °C; pH 7.4; haematocrit=20%; $[Ca^{2+}]=0.5$ mM

At the end of the procedure 40 to 90% of the added isotopes was found inside the cells. Conceivably, the loading was the more effective, the higher specific activities were used and the shorter was the time required for washings.

Characterization of ^{22}Na-Loaded Red Cells

In ^{22}Na-loaded red cells the alkali cation composition, ATP and 2,3-DPG concentrations did not differ significantly from the values measured in untreated cells. The biconcave shape of the cells was completely preserved (Table 1).

Neither the passive, nor the ouabain-sensitive K^+ transport was altered by the nigericin or the gramicidin D treatment. Total K^+ influx

Table 1. Effect of the cation-loading procedures on the cation composition, metabolic state and morphology of red cells[a]

Parameter examined	Normal fresh red cells, $n=12$	^{22}Na-loaded red cells, $n=6$ (nigericin-loading)	Difference	Ca^{2+}-loaded[b] red cells, $n=6$ (A 23187-loading)	Difference
$[K^+]$ intracellular mmole/liter of cells	107.5 ±6.8[c]	109.4 ±5.5	N.S.[d]	110 ±8.5	N.S.
$[Na^+]$ intracellular mmole/liter of cells	8.7 ±1.5	9.3 ±2.1	N.S.	9.1 ±1.3	N.S.
ATP mmole/liter of cells	1.15±0.18	1.17±0.23	N.S.	1.21±0.14	N.S.
2,3-DPG mmole/liter of cells	3.95±0.56	3.83±0.85	N.S.	4.01±0.71	N.S.
Morphological index (I_m)	100	100	∅	3.4 ±2.1	$p<0.01$

[a] Cells were loaded with cations according to the procedures described in Materials and Methods.
[b] 0.2–1.0 mM internal $[Ca^{2+}]$.
[c] Mean ± SD values.
[d] N.S. = not significant.

from isosmotic NaCl medium containing 10 mM KCl and 2.5 mM $MgCl_2$ was 48.2 ± 9.4 μmoles/liter of cells/min in control cells, and 52.4±12.1 μmoles/liter of cells/min in ionophore-treated cells. The ouabain-sensitive influx values were 28.2±6.3, and 30.4±5.7 μmoles/liter of cells/min, respectively (mean ± SD values, $n=6$ in both cases).

In the first 30 min the extrusion rate of ^{22}Na was maximal even in the absence of external substrate. Addition of 5 mM inosine resulted in a further continuous unchanged sodium efflux up to at least 90 min. Fig. 3 demonstrates the Na^+ pump activity in ^{22}Na-loaded cells without added substrate. Na^+ efflux was inhibited by 10^{-5} M ouabain and 10 μg/ml oligomycin to a similar extent, whereas 0.2 mM $LaCl_3$ had no significant effect on the Na^+ pump.

Characterization of Ca^{2+}-Loaded Red Cells

Red cells containing 0.01–5.0 mM Ca^{2+} could be obtained by the technique described.

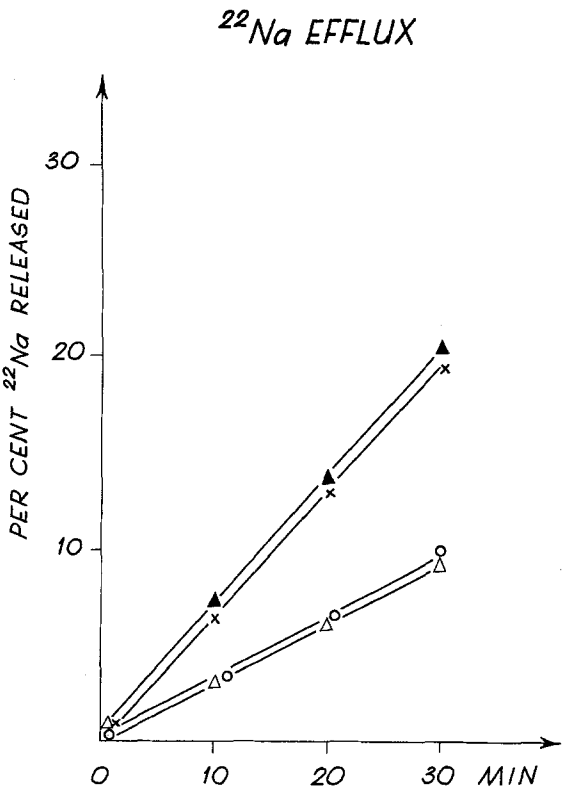


Fig. 3. ^{22}Na efflux from loaded intact erythrocytes. Medium: 0.15 M NaCl+10 mM KCl. Initial $[Na^+]_i$: 8.5 mmole/liter RBC. Initial radioactivity: 46,000 cpm/ml RBC. $t=37$ °C; pH 7.4; haematocrit=5%. ▲—▲ control; ×—× 0.2 mM $LaCl_3$; △—△ 10 μg/ml oligomycin; ○—○ 10 μM ouabain

The ATP and 2,3-DPG contents of the Ca^{2+}-loaded cells were not altered (Table 1). However, due to the high intracellular Ca^{2+} concentration the K^+ permeability of the red cell membrane remained high. Therefore, to avoid the consequences of K^+ efflux, Ca^{2+} flux experiments were carried out in KCl media. The complete elimination of the Ca^{2+} ionophore by albumin was checked by measuring ^{45}Ca influx into normal and ionophore-treated cells. The influx of ^{45}Ca was immeasurably low in both cases, which indicates that the ionophore-treated cells regained their low Ca^{2+} permeability.

In Ca^{2+} pump studies the initial Ca^{2+} efflux rate was found to be constant from 0.2 to 5.0 mM internal Ca^{2+} concentration (Fig. 4*a*). In this range the mean Ca^{2+} efflux rate was 86.2 ± 5.6 μmoles/liter of cells/min (37 °C; ±SD values; $n=24$). Up to 0.2 mM intracellular $[Ca^{2+}]$ the rate of efflux markedly increased with the Ca^{2+} concentration.

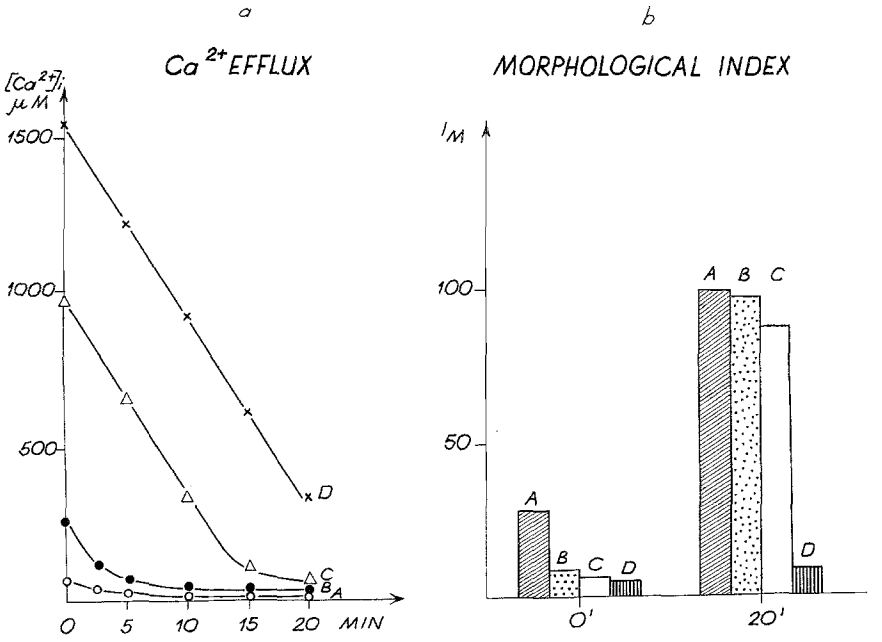


Fig. 4. Ca^{2+} efflux (*a*) and change in the morphological index (*b*) of Ca^{2+}-loaded intact red cells. Medium: 0.16 M KCl + 0.5 mM $CaCl_2$ + 5 mM inosine. t = 37 °C; haematocrit = 4%. $[Ca^{2+}]_i$ at the beginning of the experiment: *A*: 75 μM; *B*: 260 μM; *C*: 975 μM; *D*: 1,550 μM

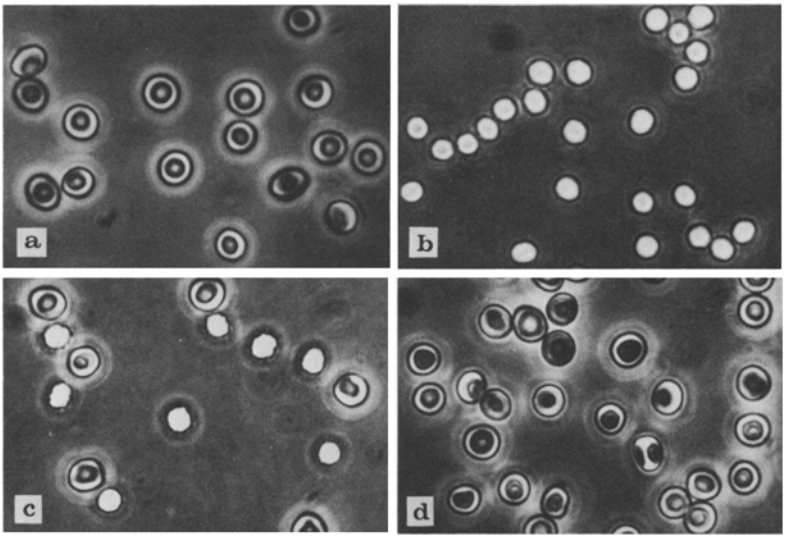


Fig. 5. The shape of red cells under phase-contrast microscope. (*a*) Before ^{45}Ca loading; (*b*) After ^{45}Ca loading ($[Ca^{2+}]_i$ = 440 μM); (*c*) During Ca^{2+} pumping ($[Ca^{2+}]_i$ = between 10 and 50 μM); (*d*) After restoration ($[Ca^{2+}]_i$ = below 5 μM) (for details *see text*)

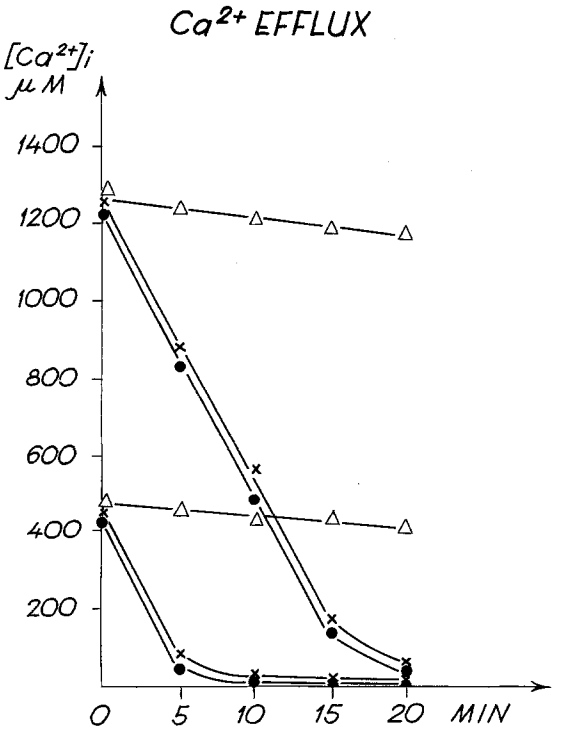


Fig. 6. Effect of ouabain and $LaCl_3$ on the Ca^{2+} efflux from Ca^{2+}-loaded intact red cells. Two cell populations with different initial intracellular Ca^{2+} concentrations (480 μM and 1,260 μM) were examined. Medium: 0.16 M KCl+2 mM $CaCl_2$+5 mM inosine. t=37 °C; pH 7.4; haematocrit=4%. ×—× control; ●—● 10 μM ouabain; △—△ 0.2 mM $LaCl_3$

During incubation at 37 °C, in the presence of 5 mM inosine the intracellular Ca^{2+} concentration fell below 10 μM even if the initial internal [Ca^{2+}] was higher than 1.0 mM. External Ca^{2+} (up to 2.0 mM) had no effect on this Ca^{2+} extrusion.

The shape of Ca^{2+}-loaded red cells depended on their intracellular Ca^{2+} concentration. Above 200 μM $[Ca^{2+}]_i$ the overwhelming majority of the cells were microspherocytes. During the course of Ca^{2+} pumping, at about 10 μM intracellular [Ca^{2+}] the original shape was restored (Figs. 4*a* and 5). The regeneration of biconcave shape was almost complete even at an initial intracellular Ca^{2+} concentration higher than 1.0 mM and was not affected by external Ca^{2+}. After the removal of Ca^{2+} the low K^+ permeability of the cells was also restored; moreover, they exhibited ouabain-sensitive K^+ uptake.

The Ca^{2+} efflux rate was highly temperature-dependent. By increasing the temperature from 22 to 37 °C a 3.8-fold acceleration of efflux was measured.

The rate of Ca^{2+} efflux was not affected by 10^{-5} M ouabain or 10 μg/ml oligomycin. Addition of 0.2 mM $LaCl_3$ to the incubation medium resulted in an instantaneous and almost complete inhibition of the Ca^{2+} efflux (Fig. 6).

Discussion

Cation-Loading Techniques

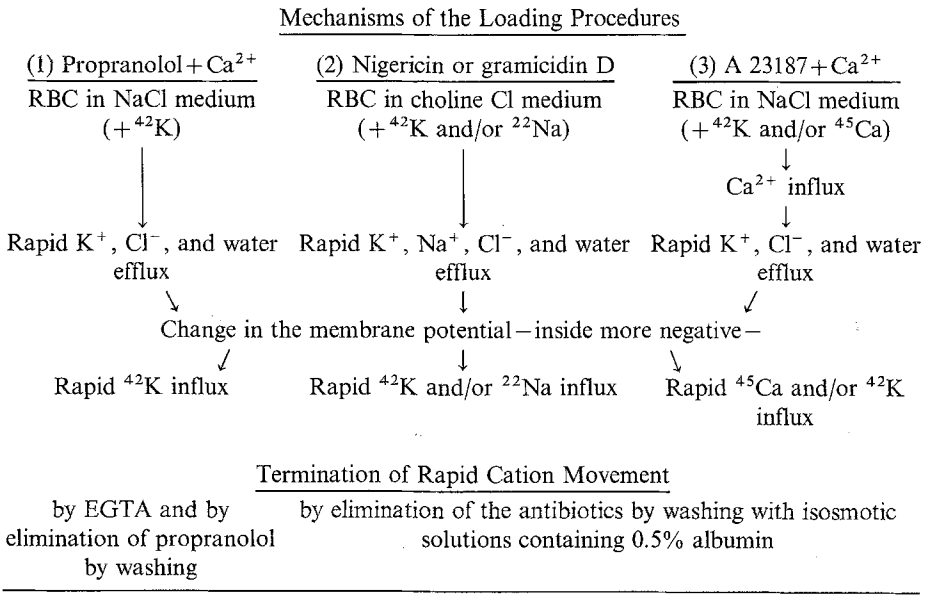
Accompanying the propranolol + Ca^{2+}-induced K^+ loss, as a result of the change in the membrane potential, a rapid tracer K^+ influx can be observed (Ekman *et al.*, 1969; Glynn & Warner, 1972). By terminating this process in the first minutes with Ca^{2+}-chelating agents, e.g. EGTA, and eliminating propranolol by several washings, red cells enriched in ^{42}K can be obtained.

In active cation pump studies, however, loading with labelled Na^+ or Ca^{2+} is of greater importance. It is shown in this paper that the above strategy can also be applied to this end: by the aid of ionophores a rapid Na^+ or Ca^{2+} uptake can be achieved by altering the membrane potential. The proposed scheme for the mechanism of cation loading by means of propranolol + Ca^{2+}, nigericin or gramicidin D, and A 23187 + Ca^{2+}, is presented in Table 2.

By the techniques described, not only minute amounts of isotopes can be introduced into the cells. The intracellular Na^+ level can be varied over a wide range as in the method of Cass and Dalmark (1973) by increasing the Na^+ concentration in the loading medium and by prolonging the loading time. The intracellular Ca^{2+} concentration can also be elevated to the millimolar level without any cell injury.

The termination of ionophore-catalyzed cation loading procedures is based on the high ionophore-binding capacity of albumin (Gárdos, 1972). At 0 °C the ion transport processes are slowed down, whereas the adsorption of the ionophores to albumin becomes stronger. This rapid and efficient elimination of the ionophores enables us to study active Na^+ and Ca^{2+} efflux in intact red cells.

Table 2



Cation Pump Studies

As the ^{22}Na-loading method does not alter the metabolic state and membrane characteristics of red cells, the study of the Na^+ pump in intact cells loaded with ^{22}Na by the ionophore technique became possible. Stoichiometric studies on Na^+ transport are under way.

As for the Ca^{2+} pump ample evidence has been presented for its existence in red cells connected with the $(Ca^{2+} + Mg^{2+})$-activated ATPase of the membrane (Schatzmann, 1966; Lee & Shin, 1969; Olson & Cazort, 1969; Schatzmann & Vincenzi, 1969; etc.). The elucidation of the mechanism of this transport process, however, needs further investigation. The Ca^{2+}-loaded intact cells described above seem to be very suitable for such studies, since by this technique intact red cells containing the required amount of Ca^{2+} and its radioactive isotope can be obtained. In Ca^{2+} extrusion experiments with resealed ghosts (Schatzmann, 1973; Quist & Roufogalis, 1975), or PCMBS-treated red cells (Schatzmann, 1973) a considerable amount of retained Ca^{2+} was found. In contrast, cells prepared by the ionophore-method were able to reduce the intracellular $[Ca^{2+}]$ to about 1 μM; i.e. the normal intracellular Ca^{2+} concentration (Harrison & Long, 1968; Lichtman & Weed, 1973), even

if the initial $[Ca^{2+}]_i$ was higher than 1.0 mM. It appears that Ca^{2+} retention is the consequence of cell injury caused by the various treatments.

The high efficiency of the Ca^{2+} pump is due to the fact that already very low amounts of intracellular Ca^{2+} activate the specific transport system. Schatzmann (1973) has demonstrated that in resealed ghosts the Ca^{2+} pump works with its full capacity at 10^{-5} M internal $[Ca^{2+}]$. The Ca^{2+} concentration that activates $(Ca^{2+}+Mg^{2+})$-ATPase in red cell membrane fragments is also around this value (Wolf, 1972; Schatzmann, 1973). In our experiments the rate of Ca^{2+} efflux was maximal only at internal Ca^{2+} concentration greater than 200 μM. The difference with respect to ghost preparations can be attributed to the high intracellular chelator content of the intact cell, which competes with the Ca^{2+} receptors of the pump system. Further experiments are needed to characterize precisely the activation of the Ca^{2+} pump by internal Ca^{2+} in intact red cells.

The maximal rate of Ca^{2+} extrusion found in our experiments (about 85 μmoles/liter of cells/min) was lower than that measured by Schatzmann (1973) in resealed ghosts (about 150 μmoles/liter of cells/min), but was higher than the values of Quist and Roufogalis (1975) (about 40 μmoles/liter of cells/min)[1]. As the rate of Ca^{2+} influx into extensively ATP-depleted red cells at 2 mM external $[Ca^{2+}]$ is about 0.02–0.2 μmoles/liter of cells/min (Lew, 1974), the Ca^{2+} pump apparently works under physiological conditions with not more than about 0.1% of its full capacity.

A selective inhibitor of the Ca^{2+} pump would greatly facilitate stoichiometric studies. Whereas SH-reagents seem to be unsuitable (*see* Schatzmann & Vincenzi, 1969), lanthanides may prove useful in this respect. Schatzmann and Tschabold (1971) reported the complete inhibition of Ca^{2+} extrusion from red cell ghosts by 1.0 mM Ho^{3+} and Pr^{3+}. A similar effect was detected by Quist and Roufogalis (1975) with 0.1 mM La^{3+}. In our experiments 0.2 mM external La^{3+} caused a rapid and almost complete inhibition of Ca^{2+} efflux from intact red cells. This lanthanum concentration had no effect on the active and passive alkali cation fluxes. As the penetration of trivalent cations into the intact cells is presumably very slow (Weiss, 1974), the inhibitory effect seems to be exerted from the external side of the cell membrane.

In contrast to the Ca^{2+} ionophore treatment of red cells reported by White (1974), our procedure proved to be completely reversible even

1 Calculated from the data available in their paper.

from the morphological point of view. The most conspicuous features of the morphological changes were, as compared to the shape changes induced by energy depletion or drugs, the very rapid transition between the two final stages, the microspherocyte and the biconcave disk, and the very low number of transient forms (echinocytes, echino-spherocytes) either in disk-sphere transformation or its reversal. This is the morphological manifestation of the rapid changes in intracellular Ca^{2+} concentration that take place during the two processes: the ionophore-mediated Ca^{2+} uptake and Ca^{2+} extrusion by the Ca^{2+} pump.

Thanks are due to Mrs. Eva Irmai, Miss Eva Mészáros and Mrs. Susan Andrási for precise and skillful technical assistance.

References

Bodemann, H., Passow, H. 1972. Factors controlling the resealing of the membrane of human erythrocyte ghosts after hypotonic hemolysis. *J. Membrane Biol.* **8**:1

Cass, A., Dalmark, M. 1973. Equilibrium dialysis of ions in nystatin-treated red cells. *Nature, New Biol.* **244**:47

Dunn, M.J. 1974. Red blood cell calcium and magnesium: Effects upon sodium and potassium transport and cellular morphology. *Biochim. Biophys. Acta* **352**:97

Ekman, A., Manninen, V., Salminen, S. 1969. Ion movements in red cells treated with propranolol. *Acta Physiol. scand.* **75**:333

Gárdos, G. 1972. Ion transport across the erythrocyte membrane. *Haematologia* **6**:237

Gárdos, G., Sarkadi, B., Szász, I. 1975. Effect of the Ca-ionophore A 23187 on the K-transport of human red cells. *Abstr. Vth Int. Biophys. Congr.* p. 100

Gárdos, G., Szász, I., Árky, I. 1966. Structure and function of erythrocytes. I. Relation between the energy metabolism and the maintenance of biconcave shape of human erythrocytes. *Acta Biochim. Biophys. Acad. Sci. Hung.* **1**:253

Garrahan, P.J., Glynn, I.M. 1967. The behaviour of the sodium pump in red cells in the absence of external potassium. *J. Physiol.* **192**:159

Garrahan, P.J., Rega, A.F. 1967. Cation loading of red blood cells. *J. Physiol.* **193**:459

Glynn, I.M. 1956. Sodium and potassium movements in human red cells. *J. Physiol.* **134**:278

Glynn, I.M., Warner, A.E. 1972. Nature of the calcium dependent potassium leak induced by (+)-propranolol, and its possible relevance to the drug's antiarrhythmic effect. *Brit. J. Pharmacol.* **44**:271

Gunn, R.B., Tosteson, D.C. 1971. The effect of 2,4,6-trinitro-*m*-cresol on cation and anion transport in sheep red blood cells. *J. Gen. Physiol.* **57**:593

Harris, E.J., Prankerd, T.A.J. 1953. The rate of sodium extrusion from human erythrocytes. *J. Physiol.* **121**:470

Harrison, D.G., Long, C. 1968. The calcium content of human erythrocytes. *J. Physiol.* **199**:367

Lee, K.S., Shin, B.Ch. 1969. Studies on the active transport of calcium in human red cells. *J. Gen. Physiol.* **54**:713

Lew, V.L. 1974. On the mechanism of the Ca-induced increase in K permeability observed in human red cell membranes. *In:* Comparative Biochemistry and Physiology of Transport. L. Bolis, K. Bloch, S.E. Luria, and F. Lynen, editors. p. 310. North-Holland Publishing Co., Amsterdam-London

Lichtman, M.A., Weed, R.J. 1973. Divalent cation content of normal and ATP-depleted erythrocytes and erythrocyte membranes. *In:* Red Cell Shape. M. Bessis, R.I. Weed, and P.F. Leblond, editors. p. 79. Springer-Verlag, New York

Lubowitz, H., Whittam, R. 1969. Ion movements in human red cells independent of the sodium pump. *J. Physiol.* **202**:111

Olson, E.J., Cazort, R.J. 1969. Active calcium and strontium transport in human erythrocyte ghosts. *J. Gen. Physiol.* **53**:311

Quist, E.E., Roufogalis, B.D. 1975. Determination of the stoichiometry of the calcium pump in human erythrocytes using lanthanum as a selective inhibitor. *FEBS Lett.* **50**:135

Reed, P.W. 1973. Calcium-dependent potassium efflux from rat erythrocytes incubated with antibiotic A 23187. *Fed. Proc.* **32**:635 (Abstr.)

Reed, P.W., Lardy, H.A. 1972. A23187: A divalent cation ionophore. *J. Biol. Chem.* **247**:6970

Schatzmann, H.J. 1966. ATP-dependent Ca^{++} extrusion from human red cells. *Experientia* **22**:364

Schatzmann, H.J. 1973. Dependence on calcium concentration and stoichiometry of the calcium pump in human red cells. *J. Physiol.* **235**:551

Schatzmann, H.J., Tschabold, M. 1971. The lanthanides Ho^{3+} and Pr^{3+} as inhibitors of calcium transport in human red cells. *Experientia* **27**:59

Schatzmann, H.J., Vincenzi, F.F. 1969. Calcium movements across the membrane of human red cells. *J. Physiol.* **201**:369

Schwoch, G., Passow, H. 1973. Preparation and properties of human erythrocyte ghosts. *Mol. Cell. Biochem.* **2**:197

Szász, I., Gárdos, G., Árky, I. 1971. Study of ATP-consuming functions of erythrocytes during blood preservation. *Bibl. Haematol. (Basel)* **38**:196

Weiss, G.B. 1974. Cellular pharmacology of lanthanum. *Annu. Rev. Pharmacol.* **14**:343

White, J.G. 1974. Effects of an ionophore, A23187, on the surface morphology of normal erythrocytes. *Am. J. Pathol.* **77**:507

Whittam, R., Ager, M.E. 1965. The connexion between active cation transport and metabolism in erythrocytes. *Biochem. J.* **97**:214

Wolf, H.U. 1972. Studies on a Ca^{2+}-dependent ATPase of human erythrocyte membranes. Effects of Ca^{2+} and H^{+}. *Biochim. Biophys. Acta* **266**:361