Acta vet. scand. 2001, *42,* 261-270.

# Electrolyte Composition of Mink (*Mustela vison*) Erythrocytes and Active Cation Transporters of the Cell Membrane

By *O. Hansen* and *T. N. Clausen*

Department of Physiology, Aarhus University, Århus, and Danish Fur Breeders' Research Centre, Tvis, Holstebro, Denmark.

**Hansen O, Clausen TN: Electrolyte composition of mink (*Mustela vison*) erythrocytes and active cation transporters of the cell membrane. Acta vet. scand. 2001, 42, 261-270.** – Red blood cells from mink (*Mustela vison*) were characterized with respect to their electrolyte content and their cell membranes with respect to enzymatic activity for cation transport. The intra- and extracellular concentrations of $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$ and $Mg^{2+}$ were determined in erythrocytes and plasma, respectively. Plasma and red cell water content was determined, and molal electrolyte concentrations were calculated. Red cells from male adult mink appeared to be of the low-$K^+$, high-$Na^+$ type as seen in other carnivorous species. The intracellular $K^+$ concentration is slightly higher than the extracellular one and the plasma-to-cell chemical gradient for $Na^+$ is weak, though even the molal concentrations may differ significantly. Consistent with the high intracellular $Na^+$ and low $K^+$ concentrations, a very low or no ouabain-sensitive $Na^+$,$K^+$-ATPase activity and no $K^+$-activated pNPPase activity were found in the plasma membrane fraction from red cells. The $Cl^-$ and $Mg^{2+}$ concentrations expressed per liter cell water were significantly higher in red cells than in plasma whereas the opposite was the case with $Ca^{2+}$. The distribution of $Cl^-$ thus does not seem compatible with an inside-negative membrane potential in mink erythrocytes. In spite of a steep calcium gradient across the red cell membrane, neither a calmodulin-activated $Ca^{2+}$-ATPase activity nor an ATP-activated $Ca^{2+}$-pNPPase activity were detectable in the plasma membrane fraction. The origin of a supposed primary $Ca^{2+}$ gradient for sustaining of osmotic balance thus seems uncertain.

***erythrocytes; plasma; electrolytes; red cell; mink red cells; $Na^+$,$K^+$-ATPase; membrane potential; osmotic balance; PM-CaATPase.***

## Introduction

The plasma membrane-embedded ($Na^+$+$K^+$)-activated ATPase (Na,K-ATPase, EC 3.6.1.37) of mammalian cells is usually supposed to have an essential role in counterbalancing passive ionic leaks and oncotic forces from intracellular proteins and fixed phosphate groups, i.e. in cell volume regulation (*Dunham & Hoffman* 1980, *Macknight & Leaf* 1980). There are, however, a few exceptions from this general principle, in which case a plasma membrane-bound $Ca^{2+}$-ATPase and a $Na^+/Ca^{2+}$-exchange mechanism are usually supposed to have similar roles (*Parker* 1973, 1979, *Parker et al.* 1975).

It has been known for years that red blood cells in some mammalian species may be devoid of Na,K-ATPase and yet be able to maintain ionic balance and cell volume. Some carnivorous species, e.g. the cat and the dog, have low-potassium erythrocytes due to a lack of plasma membrane Na,K-ATPase (*Bernstein* 1954, *Chan et al.* 1964) and $Na^+/Ca^{2+}$ exchange may

partly account for cell volume maintenance (*Parker* 1973, 1979, *Parker et al.* 1975). Also red cells from ferrets *(Mustela putorius furo),* i.e. a Mustelidae species belonging to a collateral branch of the carnivorous phylogenetic tree have high sodium and low potassium content (*Flatman & Andrews* 1983, *Milanick* 1989). In other species, e.g. sheep and goat, the eythrocytes may be of a high-potassium or a low-potassium type (*Evans & Phillipson* 1957). In the latter case the number of sodium pumps per red cell may be reduced or, more likely, the Na,K-ATPase activity is inhibited by a membrane-bound inhibitory factor closely related to the blood group L antigen (*Tucker et al.* 1976). The $K^+$ concentration is relatively low but not that low as seen in carnivorous species.

To our knowledge, red cells from the only carnivorous species used for large-scale animal production, the domestic mink *(Mustela vison),* were never characterized with respect to electrolyte composition. In this study the ionic type of red blood cells of the domestic mink is characterized, and moreover, the plasma membrane of mink red cells with respect to the main ion-transporting ATPases: The ($Na^+$+$K^+$)-activated ATPase and the $Ca^{2+}$-activated ATPase (PM-$Ca^{2+}$ATPase).

## Materials and methods

*Preparation of plasma, red cell contents and erythrocyte plasma membranes.*

Domestic mink *(Mustela vison)* from a fur research farm free of plasmacytosis were used in this study. Twelve adult male mink selected for pelting at the end of the mating season in 1998 were anaesthetized by means of an intraperitonal injection of pentobarbital (25 mg/kg). Another 12 adult male mink (1999a) and 12 adolescent (7 months) male mink were sacrificed for follow-up studies (1999b). About 10 ml of blood was obtained by heart puncture from each animal. The blood was stabilized by collection in heparinized tubes, handled and transported at 0-2 °C for about 2 h and then rewarmed and kept at room temperature before separation. Plasma was obtained after separation for 5 min at 1600 g (Heraeus Microfuge 1.0). The intermediary layer (buffy coat) was carefully withdrawn and discarded. After resuspension to the original volume in 0.9% NaCl the erythrocyte fraction was washed 3 times by sedimentation at 1600 g for 5 min. Finally the erythrocyte fraction was suspended in 300 mM sucrose (final volume 25 ml) and washed by sedimentation at 20,000 g (Beckman, rotor 50.2 Ti). The supernatant was carefully withdrawn and discarded. 250 $\mu$l of the packed erythrocytes were withdrawn for determination of dry matter. The remaining volume of packed erythrocytes was weighed (about 3 g), suspended in exactly 6 ml of a medium containing 20 mM imidazole + 0.5 mM EDTA (pH 7.4, adjusted with $HNO_3$) for hemolysis and centrifuged for 15 min at 35,000 g (Beckman, rotor 70.1 Ti). Supernatant was withdrawn for determination of $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$ and $Mg^{2+}$. The sediment was resuspended in 25 ml of the imidazole/EDTA buffer and washed twice by precipitation at 35,000 g for 15 min, then twice in 20 mM imidazole and finally once in 40 mM imidazole + 40 mM histidine (pH 7.1). The individual sediments were pooled, resuspended in the same buffer and homogenized in a tightly fitting Teflon glass homogenizer surrounded by an ice bath. The final product, the cell membrane fraction, was stored at -20 °C until determination of enzymatic activity.

In one series (1999b) a possible release or uptake of electrolytes during washing was determined in the following way: All supernatants from washings were recovered, weighed and used for determination of $Na^+$, $K^+$, $Cl^-$ and $Mg^{2+}$. At each step during washing the weight of the precipitate including residual plasma, saline or sucrose was determined. The differ-

ence between this weight and the original weight of packed erythrocytes was taken as contaminating plasma, saline or sucrose. In this way, step-by-step transfer of electrolytes between erythrocytes and plasma could be calculated and accounts of step-by-step and net efflux or influx of electrolytes made. Due to contamination by $Ca^{2+}$ of redistilled water and reagents, a similar assessment of $Ca^{2+}$ release or uptake by erythrocytes during washing was not undertaken.

*Measurements on plasma, saline and sucrose used for washing, and on erythrocyte contents (lysate).*

Dry matter of plasma and erythrocyte fraction was determined by heating at 80 °C until constant weight. Molar concentrations of $Na^+$ and $K^+$ were determined using a Radiometer (Copenhagen, Denmark) FLM3 flame photometer with lithium as internal standard. $Ca^{2+}$ and $Mg^{2+}$ were determined by atomic absorption spectrophotometry (Philips PU 9200; Pye Unicam, Cambridge, UK). Aliquots of plasma and erythrocyte content were adequately diluted and compared with standards of $CaCl_2$ (6.25-50 $\mu$M) with addition of 0.2% (w/v) KCl or with standards of $MgCl_2$ (10-400 $\mu$M). Determination of chloride was carried out with an ABU91 Autoburette from Radiometer in which 1 mM $AgNO_3$ was titrated with 1 mM NaCl for calibration. (Data on intracellular $Cl^-$ in 1998 are missing due to adjustment of the imidazole/EDTA buffer used for cell lysis with HCl). In control experiments it was shown that addition of bovine hemoglobin (Sigma) corresponding to an estimated concentration in lysate from mink erythrocytes (0.1 g/ml) did not influence chloride determination and neither did albumin in plasma. Calculation of molal concentrations of $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$ and $Cl^-$ was carried out by dividing the molar concentrations with $(1\text{-}f_d)$ where $f_d$ is the fraction of dry matter.

*Enzymatic activities of erythrocyte plasma membrane fraction.*

ATPase activities were determined at 37 °C by the coupled assay utilizing the NADH/$NAD^+$ conversion in the presence of auxiliary enzymes (*Nørby* 1988). $Na^+$,$K^+$-ATPase determined in the absence and the presence of $10^{-3}$ M ouabain was supposed to represent total and basal (~unspecific $Mg^{2+}$-ATPase) hydrolytic activity, respectively. The $K^+$-activated hydrolysis of the artificial substrate pNPP ($K^+$-pNPPase) was assayed as described elsewhere (*Hansen* 1992). The activity obtained by substitution of $K^+$ with $Na^+$ was taken to represent unspecific activity. Total and basal hydrolytic activity related to $Ca^{2+}$-ATPase were determined at 0.1 mM $Ca^{2+}$ and 1 mM EDTA, respectively. Calmodulin (phosphodiesterase 3':5'-cyclic nucleotide activator from Sigma) at 80 nM was preincubated with the membrane fraction for 5 min before addition of $Ca^{2+}$ and substrate (*Foder & Scharff* 1981). $Ca^{2+}$-pNPPase activity was determined in the presence and absence of 0.5 mM ATP.

**Results**

In Table 1 are shown the molar as well as the molal concentrations in mink plasma and erythrocytes of $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$ and $Mg^{2+}$. The corrections for dry matter were carried out on the individual values which explains an apparent inconsistency by conversion to mean molal concentrations.

It is seen that the intracellular concentration of $K^+$ is very low and apparently lower than the concentration in plasma (see below), whereas the intracellular concentration of $Na^+$ is nearly as high as the extracellular one. A significant difference in $Na^+$ concentrations intra- and extracellularly may, however, exist, at least according to data obtained in 1999. The intracellular molal concentrations of $Cl^-$ and $Mg^{2+}$ are significantly higher than the respective extra-

Table 1. Dry matter and electrolyte concentrations in plasma and erythrocytes before (*first column:* mmoles per l plasma or per kg erythrocytes) and after correction for dry matter (*second column:* mmoles per kg $H_2O$). Values are ± SEM.

| | | | Dry matter % | $Na^+$ | | $K^+$ | | $Cl^-$ | |
|---|---|---|---|---|---|---|---|---|---|
| | 1998 | (n=12) | 7.81±0.16 | 151.5±1.3 | 164.3±1.4 | 3.9±0.3 | 4.4±0.3# | 102.5±1.3 | 111.1±1.3 |
| Plasma | 1999a | (n=12) | 8.56±0.12 | 152.3±0.5 | 166.7±0.5** | 4.2±0.0 | 4.6±0.0** | 99.7±1.5 | 109.0±1.7** |
| | 1999b | (n=12) | 7.88±0.10 | 152.1±0.4 | 165.2±0.4** | 3.8±0.1 | 4.1±0.1* | 112.5±1.0 | 121.9±1.0** |
| | 1998 | (n=11) | 38.75±0.72 | 98.2±4.9 | 160.7±8.3 | 2.2±0.3 | 3.5±0.4# | | |
| Erythr. | 1999a | (n=12) | 41.49±0.21 | 83.2±1.8 | 142.3±3.0** | 1.1±0.1 | 1.9±0.1** | 98.6±2.9 | 168.6±5.1** |
| | 1999b | (n=12) | 42.51±0.30 | 75.6±2.1 | 131.4±4.2** | 2.0±0.1 | 3.5±0.1* | 82.8±2.6 | 144.1±4.7** |

Table 1. Continued.

| | | | $Ca^{2+}$ | | $Mg^{2+}$ | |
|---|---|---|---|---|---|---|
| | 1998 | (n=12) | 1.89±0.03 | 2.05±0.04** | 0.88±0.05 | 0.95±0.06** |
| Plasma | 1999a | (n=12) | 2.11±0.04 | 2.31±0.04** | 1.33±0.02 | 1.45±0.02** |
| | 1999b | (n=12) | 2.47±0.02 | 2.68±0.02** | 1.11±0.02 | 1.21±0.02** |
| | 1998 | (n=11) | 0.086±0.006 | 0.138±0.010** | 2.98±0.15 | 4.92±0.30** |
| Erythr. | 1999a | (n=12) | 0.052±0.006 | 0.088±0.010** | 3.89±0.20 | 6.64±0.35** |
| | 1999b | (n=12) | 0.098±0.004 | 0.171±0.006** | 4.01±0.25 | 6.97±0.43** |

Plasma vs. erythrocytes same year: # p>0.10 * P<0.01 **P<0.001

cellular concentrations. For $Ca^{2+}$ an opposite directed concentration gradient exists.

Flux data during washing of the red cells were obtained in one of the series (1999b). In Table 2 are shown net fluxes of $Na^+$, $K^+$, $Cl^-$ and $Mg^{2+}$ in saline plus sucrose used for washing of the erythrocytes before lysis. The accumulated values for net efflux are the result (not shown) of a continuous leak of $K^+$ at each step of washing, a moderate influx of $Na^+$ during saline incubation and a prevailing efflux during sucrose incubation, some influx of $Cl^-$ in saline (probably counterbalanced by $HCO_3^-$ efflux) and a larger efflux in sucrose, and finally hardly any efflux

Table 2. Accumulated values of electrolytes from 4 x washing and in the final lysate from erythrocytes (1999b). An estimated value for the sum in molal concentration is given in the last column. Number of observations in brackets.

| | 4 x washing | lysate | total | |
|---|---|---|---|---|
| | mmoles per kg red cells ± SEM | | | mmol/kg $H_2O$ |
| $Na^+$ | 9.76±3.01 (11) | 75.58±2.12 (12) | 85.34±3.68 | 148.4 |
| $K^+$ | 4.11±0.10 (11) | 2.00±0.08 (12) | 6.11±0.13 | 10.6 |
| $Cl^-$ | 2.20±3.27 (12) | 82.78±2.55 (12) | 84.98±4.15 | 147.8 |
| $Mg^{2+}$ | 0.25±0.02 (10) | 4.01±0.25 (12) | 4.26±0.25 | 7.4 |

Table 3. Hydrolytic activities of mink erythrocyte membrane fraction, $(Na^+ + K^+)$-activated ATPase activity in the absence and the presence of ouabain, pNPPase activity in the presence of $K^+$ or $Na^+$, $Ca^{2+}$-activated ATPase activity in the presence of $Ca^{2+}$ or EDTA ± calmodulin and pNPPase activity in the presence of $Ca^{2+}$ ± ATP. Number of determinations in brackets.

| | 1998 | 1999 |
|---|---|---|
| | nmol·(mg protein)$^{-1}$·min$^{-1}$ ± SEM | |
| $(Na^+ + K^+)$-ATPase | 18.4±0.9 (9) | 15.6±2.3* (7) |
| (Na++K+)-ATPase + ouabain | 14.5±2.1 (7) | 9.6±1.4* (7) |
| $K^+$-pNPPase | 12.7±0.8 (4) | 11.3±1.4 (3) |
| $Na^+$-pNPPase | 11.6±2.1 (3) | 10.2±0.2 (3) |
| $Ca^{2+}$-ATPase | | |
| Activity in the presence of $Ca^{2+}$ | 22.5±1.2 (7) | n.d. |
| Activity in the presence of $Ca^{2+}$+calmodulin | 19.8±1.8 (7) | 37.8±12.8 (4) |
| Activity in the presence of EDTA | 17.3±1.6 (5) | n.d. |
| Activity in the presence of EDTA+calmodulin | 24.6±1.9 (5) | 23.4±4.7 (4) |
| $Ca^{2+}$-pNPPase (- ATP) | 7.4±0.1 (3) | n.d. |
| $Ca^{2+}$-pNPPase (+ ATP) | 6.4±0.1 (3) | n.d. |

n.d. = not determined. * $P<0.05$

of $Mg^{2+}$ at any step. It is seen that the main conclusions on electrolyte concentrations of mink erythrocytes as derived from Table 1 are not seriously invalidated by data on electrolyte fluxes during washing of the red cells. The intracellular $Na^+$ and $Cl^-$ concentrations are relatively unchanged by accounts on recovery, whereas the extremely low $K^+$ concentration from Table 1 is tripled after correction for fluxes. The intracellular $K^+$ concentration is still low but apparently somewhat higher than the extracellular one. Even after corrections for fluxes during washing it still holds that mink erythrocytes are of the high-$Na^+$, low-$K^+$ type.

Sodium pump related hydrolytic activities of the erythrocyte membrane fraction were measured as the ouabain-sensitive $(Na^+ + K^+)$-activated ATPase activity and as the $K^+$-activated pNPPase activity. The results are shown in Table 3. The pNPPase activity in the presence of $K^+$ or $Na^+$ did not differ significantly, and a very low, though in one of the 1999 membrane preparations significant, ouabain-sensitive Na, K-ATPase activity was seen. Mature red cells of mink thus seem to be nearly deprived of the Na,K-ATPase. A minor component of ouabain-sensitive Na,K-ATPase would be consistent with some contamination with reticulocytes in which this activity is retained.

Similarly, calcium pump related hydrolytic activities of the erythrocyte membrane fraction were measured as the calmodulin-activated $Ca^{2+}$-ATPase and as the ATP-activated $Ca^{2+}$-pNPPase activity. As also seen from Table 3 no significant increase in the two activities was seen with calmodulin or ATP. It seems therefore that mink red cells, as well as being totally deprived of Na,K-ATPase, are also deficient in calcium pump activity.

## Discussion

The aim of the present study is a characteriza-

tion of electrolytes in plasma and red cells from the only carnivorous species used for large-scale animal production, the domestic mink *(Mustela vison)*. The erythrocyte membrane is moreover characterized with respect to $(Na^+ + K^+)$- and $Ca^{2+}$-activated ATPase activity. The perspectives associated with the transmembranous concentration gradients, expressed per liter plasma water and cell water, for $Na^+$, $K^+$ and, in particular, for $Cl^-$ are also focused upon in this study. On the other hand, a more comprehensive analysis of the mink erythrocyte membrane with respect to channels and carriers for electrolyte transport is outside the scope of the present study.

It appears that erythrocytes from healthy, domestic male mink, whether adult or adolescent, are of the low-$K^+$, high-$Na^+$ type as seen in other carnivorous species and that the plasma membrane of red cells is practically devoid of ouabain-sensitive Na,K-ATPase activity. The generally accepted principle, that body cells as well as red blood cells of most mammalian species have high intracellular $K^+$ and low $Na^+$ concentrations, may have other exceptions, however. *Bookchin et al.* (2000) recently described a fraction (some 4%) of sicle cells from human beings with sicle cell anemia and an extremely low proportion of normal red cells that appeared to be of the low-$K^+$, high-$Na^+$ type.

One practical aspect of the odd electrolyte distribution between mink red cells and plasma is the following: A minor degree of hemolysis will not significantly change plasma-$K^+$, which is a parameter of clinical significance in some mink diseases (*Wamberg et al.* 1992). Another aspect is an underscore of the high plasma osmolality of mink plasma (*Wamberg et al.* 1992, *Clausen et al.* 1996), in the present study indicated by the high plasma $Na^+$ concentration, which may give rise to further investigations. Since mink blood is easily available in some countries, e.g. Canada and Denmark, during the pelting season, the red cells of this species seem ideal for further studies on osmoregulation in the absence of an active sodium pump.

The plasma concentrations of electrolytes in the 1998 study are almost the same as found in the 2 series of experiments in 1999, whereas the intracellular concentrations may differ somewhat though the same procedure was used each time. The plasma concentrations of $Na^+$, $K^+$, and $Mg^{2+}$ in all mink of the present study and of $Cl^-$ and $Ca^{2+}$ in adolescent mink (Table 1, experiment 1999b) are also almost exactly identical to those previously found in healthy mink dams (*Wamberg et al.* 1992, *Clausen et al.* 1996), whereas $Cl^-$ and $Ca^{2+}$ are somewhat lower in adult male mink (Table 1, experiments 1998 and 1999a). The high plasma-$Na^+$ concentration is consistent with a very high plasma osmolality, of the order of 310-330 mOsm, in mink as seen in previous studies (*Wamberg et al.* 1992, *Clausen et al.* 1996). The tonicity of 300 mM sucrose used for the final wash of mink red cells thus does not exceed that of erythrocytes and hypertonic cell shrinkage seems unlikely.

No correction was made for trapped sucrose in the final wash of the mink red cells with 300 mM sucrose, which may have added no more than 0.2% dry matter (0.3 M × 342 (MW) × 0.02) provided that closely packed red cells contain a maximum of 2% trapped water space (*Flatman & Andrews* 1983). A lower concentration of dry matter was found in ferret red cells but observations of considerably higher values were quoted from the literature (*Flatman & Andrews* 1983). Irrespective of a trivial correction of dry matter content for trapped sucrose (about 0.2% compared to 40% dry matter, i.e 0.5 relative per cent) and thus in calculation of red cell water content, the intracellular concentrations are dramatically increased when expressed per liter cell water.

As to the intracellular concentrations of elec-

trolytes, similar concentrations of $Na^+$ and $Mg^{2+}$ as the present ones were found in red cells from ferret by *Flatman & Andrews* (1983) when expressed per liter original cells, although they used very different media during separation. This does not hold for the $Ca^{2+}$ concentration that was 5-10 times lower and the $K^+$ concentration that was 2-3 times lower than found in the present study, the latter parameter after correction for $K^+$ efflux during washing of the red cells. Our washing procedure using isotonic NaCl and sucrose was anticipated not to be too harmful to mink erythrocyte permeability as noticed in a study with dog red cells (*Parker et al.* 1995) in which the water content was shown to be dependent on impermeant sucrose and Na+ of the media. In one series of the present experiments (Table 1, 1999b) a possible leak of electrolytes was determined (Table 2). Since the intracellular concentrations for $Na^+$ and $Cl^-$ were lower in this series than otherwise found (Table 1) a maximum leak might have taken place in this experiment. No dramatic net efflux of $Mg^{2+}$ (5.9%), $Cl^-$ (2.6%) or $Na^+$ (11.4%) was found however, whereas the intracellular $K^+$ concentration was reduced to 1/3. Even when the intracellular $K^+$ concentration is tripled the main conclusion, that mink erythrocytes are of the high-$Na^+$, low-$K^+$ type, is still valid, however.

When expressing concentrations per liter cell water a weak, though significant, chemical gradient for $Na^+$ seems to exist across the red cell membrane even after correction for efflux during washing. At a very low, inside positive, membrane potential $Na^+$ may be near equilibrium (see below). In contrast, after correction for efflux of $K^+$ during the washing procedure the intracellular concentration of this cation seems somewhat higher than the extracellular one. On the other hand, the intracellular concentration of $K^+$ in mink red cells is still far below that seen in most mammalian species.

There are few studies on the intracellular concentration of $Cl^-$ in red cells from low-$K^+$ species. Using a buffered physiological medium containing 150 mM $Cl^-$ for suspension of ferret red cells and $^{36}Cl$ as tracer *Flatman* (1987) found a ratio of 1.50 for external to internal chloride concentration, i.e. a somewhat lower intracellular chloride concentration than in the present study after separation of erythrocytes from 110-120 mM $Cl^-$ in plasma. Similarly, *Parker et al.* (1995) made an estimate of the intracellular chloride concentration in dog red blood cells by using a media containing $^{36}Cl$ and 15 min of equilibration. Somewhat lower intracellular $Cl^-$ concentrations per liter cell water were obtained by this method than in the present study at comparable external salt concentrations. Even in the absence of any corrections for dry matter the intracellular concentration of $Cl^-$ in mink erythrocytes is nearly as high as the extracellular one. Expressed per liter cell water the intracellular $Cl^-$ concentration is significantly higher than that in plasma water. After correction for membrane leak during washing of the red cells the $Cl^-$ concentration in mink red cells is nearly as high as the concentration of monovalent cations. For electroneutrality, however, a number of small intracellular electrolytes has to be taken into account in addition to the net charge of hemoglobin. In the abovementioned study on dog red cells (*Parker et al.* 1995) a net negative charge of these intracellular electrolytes and a small net negative charge of hemoglobin was calculated for counterbalancing a net positive charge from monovalent cations. A net negative membrane potential set by chloride as seen in red cells from other species (*Milanick* 1989) seems incompatible with the high intracellular concentration of this anion or the membrane potential would even have an opposite direction (inside positive). Chloride and sodium concentrations in mink plasma and erythrocytes would suggest a

membrane potential of 7-8 and 3 mV, respectively. Using an indirect method that would imply hydrogen ion equilibrium according to the membrane potential after addition of a protonophore, *Flatman & Smith* (1991) calculated a membrane potential of -10 mV in ferret red cells.

$Ca^{2+}$ is definitely not equally distributed in mink plasma and in red cells. Another divalent cation, $Mg^{2+}$, has the opposite distribution. A mechanism for extrusion of red cell $Ca^{2+}$ must exist. Provided $Na^+$ were significantly out of equilibrium a $Na^+/Ca^{2+}$-exchange mechanism might have been (part of) the explanation. Uphill $Ca^{2+}$ transport cannot be fuelled by passive $Na^+$ entry, however, in the absence of a membrane-bound Na,K-ATPase and thus a primary electrochemical gradient for this ion (*Baker* 1970). A very low and for one membrane preparation no significant ouabain-sensitive ($Na^+$+$K^+$)-activated ATPase activity and no $K^+$-activated pNPPase activity were seen in the present study. Irrespective of the ionic conditions employed, more or less the same hydrolytic activity of the cell membrane fraction was measured. This activity is thus probably due to some unspecific $Mg^{2+}$-ATPase/phosphatase associated with the erythrocyte membrane fraction. Almost the same basal $Mg^{2+}$-ATPase activity was measured in human red cells, whereas the calmodulin-activated ATPase activity was 2-3 times higher (*Foder & Scharff* 1981, *Hinds & Vincenzi* 1986). Likewise, a ouabain-sensitive ($Na^+$+$K^+$)-activated ATPase activity of $45 \pm 3$ nmol.(mg protein)$^{-1}$.min$^{-1}$ was measured in high-potassium (HK) red cells from a rare variant of a Japanese dog whereas the activity in LK cells was nil (*Maede & Inaba* 1985).

From our present knowledge and in the absence of a Na,K-ATPase and a $Na^+$ gradient the low intracellular concentration of $Ca^{2+}$ has to be due to a primary $Ca^{2+}$ pump. A $Na^+/Ca^{2+}$-exchange mechanism as found in ferret red cells (*Milanick* 1989) may then have an opposite role: extrusion of $Na^+$ for counterbalancing the oncotic forces created by internal hemoglobin. Surprisingly, we were unable to measure any $Ca^{2+}$-activated ATPase activity, irrespective of the presence of calmodulin or not, indicating no or a very low concentration of plasma membrane $Ca^{2+}$-ATPase (PM-CaATPase). Similar conclusions were reached by *Rega et al.* (1974) and by *Hinds & Vincenzi* (1986) in dog red cells though the latter authors presented indirect evidence of a calmodulin-activated $Ca^{2+}$-ATPase. When dog red cells were exposed to the ionophore A23187 in the presence of $Ca^{2+}$ a faster loss of ATP was seen (*Hinds & Vicenzi* 1986). Similarly, *Parker* (1979) showed that resealed ghosts of dog red cells were able to extrude $Ca^{2+}$, provided ATP was incorporated into them. At a low (inside negative) membrane potential and at a supposed exchange ratio of 3:1 a $Na^+/Ca^{2+}$-exchange mechanism might be effecient for extrusion of $Na^+$ driven by a $Ca^{2+}$ gradient created by an active extrusion of $Ca^{2+}$ (*Parker* 1973, 1979, *Parker et al.* 1975).

In conclusion: Mink red cells appeared to be of the low-$K^+$ type consistent with a very low or no ouabain-inhibitable $Na^+$,$K^+$-ATPase activity and no $K^+$-activated pNPPase activity. When expressed per liter water a weak plasma-to-cell concentration gradient for $Na^+$ and a weak opposite-directed $K^+$ gradient seem to exist An unexpected high intracellular $Cl^-$ concentration was found. Osmotic balance may be sustained by a primary $Ca^{2+}$ gradient the origin of which seems uncertain.

**Acknowledgment**

Thanks are due to Ms. Tove Lindahl Andersen, Ms. Edith Bjørn Møller and Mr. Toke Nørby for excellent technical assistance. This study was supported by the Danish Biomembrane Research Centre.

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## Sammendrag

*Elektrolytter i minkens røde blodlegemer og cellemembranens kationtransportører.*

I dette arbejde karakteriseres minkens røde blodlegemer, hvad angår elektrolytsammensætning, og erythrocytcellemembranen, hvad angår enzymaktivitet med relation til aktiv kationtransport. De intra- og ekstracellulære koncentrationer af $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$ and $Mg^{2+}$ i henholdsvis erythrocytter og plasma blev målt. Efter bestemmelse af vandindholdet i plasma

og erythrocytter kunne de molale elektrolytkoncentrationer i de to faser beregnes. Som hos andre kødædende pattedyrarter viste det sig, at røde blodlegemer fra voksne hanmink var af typen med lav $K^+$- og høj $Na^+$-koncentration. Den intracellulære $K^+$-koncentration er kun lidt højere end i plasma, og forskellen mellem den ekstracellulære og den intracellulære $Na^+$-koncentration er ikke stor, men alligevel signifikant, selv hvad angår de molale koncentrationer. I overensstemmelse med den høje intracellulære $Na^+$- og den lave $K^+$-koncentration måltes kun en megen lav eller slet ingen ouabain-følsom $Na^+$,$K^+$-ATPase aktivitet og ingen $K^+$-aktiveret pNPPase aktivitet i cellemembranfraktionen fra minkerythrocytter. De intracellulære $Cl^-$- og $Mg^{2+}$-koncentrationer udtrykt pr. l cellevand var signifikant højere i røde blodlegemer end i plasma, hvorimod det modsatte var tilfældet for $Ca^{2+}$. Fordelingen af $Cl^-$ i minkerythrocytter synes således ikke forenelig med en potentialforskel over cellemembranen, hvor indersiden skulle være negativ i forhold til ydersiden. Til trods for en stejl $Ca^{2+}$-gradient mellem erythrocyttens yder- og inderside var man hverken i stand til at måle en $Ca^{2+}$-ATPase aktivitet i tilstedeværelse af calmodulin eller en ATP-aktiveret $Ca^{2+}$-pNPPase aktivitet i cellemembranfraktionen. Selv om $Ca^{2+}$-gradienten må antages at være den, der sikrer osmotisk ligevægt i erythrocytten i forhold til plasma, er det derfor ikke fastslået, hvordan gradienten kommer i stand.

*(Received April 4, 2000; accepted January 23, 2001).*

Reprints may be obtained from: Otto Hansen, Department of Physiology, Aarhus University, Ole Worms Allé 160, DK-8000 Århus C, Denmark. E-mail: oh@fi.au.dk, tel: +45 89 42 28 06, fax: +45 86 12 90 65.