Magnesium metabolism in erythrocytes of various species

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Zusammenfassung

Die Gesamt- und freie Mg^{2+}-Konzentration sowie die Aktivität von Mg^{2+}-Transportsystemen wurde in Erythrozyten von 14 verschiedenen Tierarten untersucht. Obwohl die Gesamt-Mg^{2+}-Konzentration zwischen 0,5 und 4,7 mmol/l Zellen variierte, war die freie Mg^{2+}-Konzentration (gemessen mit ^{31}P-NMR) in den Erythrozyten von Ratten, Schweinen und Rindern, die einen sehr großen Unterschied in der Gesamt-Mg^{2+}-Konzentration aufweisen, nahezu gleich. Der Na^+-abhängige Mg^{2+}-Efflux über den Na^+/Mg^{2+}-Antiporter sowie der Na^+-unabhängige Efflux wies aus Mg^{2+}-beladenen Erythrozyten von Nagetieren die höchste Aktivität auf, während in Erythrozyten von Wiederkäuern die niedrigste Aktivität gefunden wurde. Der Na^+/Mg^{2+}-Antiport von kernlosen Erythrozyten war direkt mit dem K^+-Gehalt und dem Na^+/K^+-Quotienten korreliert. In kernlosen und kernhaltigen Erythrozyten war keine Korrelation des Na^+/Mg^{2+}-Antiports zum Gesamt-Mg^{2+}-Gehalt nachweisbar. Der Na^+/Mg^{2+}-Antiporter von Schweineerythrozyten konnte durch Umkehr des Na^+_i/Na^+_o-Gradienten umgekehrt werden und führte dann zum Mg^{2+}-Influx. In kernlosen Erythrozyten wird über den Na^+/Mg^{2+}-Antiporter auch Mn^{2+} in die Zellen aufgenommen, durch Mg^{2+}-Beladung der Zellen wird der Mn^{2+}-Influx über den Na^+/Mg^{2+}-Antiporter gesteigert. In (kernhaltigen) Hühnererythrozyten verläuft der Mn^{2+}-Influx nicht über den Na^+/Mg^{2+}-Antiporter und wird nicht durch Amilorid, einem Hemmstoff für den Na^+/Mg^{2+}-Antiporter, gehemmt. Im Vergleich zu anderen (kernlosen) Erythrozyten deutet dies auf eine höhere Selektivität des Na^+/Mg^{2+}-Antiporters in kernhaltigen Erythrozyten hin.

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Abstract

Total and free Mg^{2+} content and activity of Mg^{2+} transport systems were determined in erythrocytes from 14 species. Although total Mg^{2+} concentration varied between 0.5 and 4.7 mmol/l cells, free Mg^{2+} concentration measured by ^{31}P-NMR in erythrocytes from rat, pig and cattle – which express the most different total Mg^{2+} content – was nearly the same. In Mg^{2+}-loaded erythrocytes from rodents Na^+-dependent Mg^{2+} efflux via Na^+/Mg^{2+}-antiport and Na^+-independent Mg^{2+} efflux were highest, whereas in erythrocytes from ruminants both components of Mg^{2+} efflux were lowest. Na^+/Mg^{2+} antiport in non-nucleated erythrocytes was directly correlated to K^+ content and K^+/Na^+ quotient. Na^+/Mg^{2+} antiport in non-nucleated and nucleated erythrocytes was not correlated to Mg^{2+} content. Na^+/Mg^{2+} antiport in pig erythrocytes could be reversed mediating Mg^{2+} influx when the Na^+_i/Na^+_o gradient was reversed. Non-nucleated erythrocytes perform Mn^{2+} influx via Na^+/Mg^{2+} antiport. In Mg^{2+}-loaded mammalian erythrocytes Mn^{2+} influx via Na^+/Mg^{2+} antiport was enhanced. Mn^{2+} influx in nucleated chicken erythrocytes is not operating via Na^+/Mg^{2+} antiport and is not inhibited by amiloride, an inhibitor of Na^+/Mg^{2+} antiport, indicating a higher selectivity of Na^+/Mg^{2+} antiport in these cells as compared to non-nucleated erythrocytes.

Introduction

Erythrocytes from different species display significant differences in Na^+, K^+ and ATP concentration [1] as well as in the activity of Na-K-ATPase [2]. Erythrocytes from human and rodents, for example, contain high concentrations of K^+ and low concentrations of Na^+ similar to nucleated cells, whereas erythrocytes from carnivora display the reciprocal relationship. In sheep [1] and dog [3] some of the animals have a high K^+ content in their erythrocytes, whereas the rest have a low K^+ content. The low K^+ concentration of sheep and dog erythrocytes develops after birth [3]. The K^+/Na^+ ratio of erythrocytes from various species is correlated to ATP content [1].

Considerable differences were also observed in Mg^{2+} content. Erythrocyte Mg^{2+} may differ by a factor of 8 among different species [1, 4–6]. A correlation was found between erythrocyte ATP and Mg^{2+} concentration [1]. In human erythrocytes, 10 % of total Mg^{2+} is free Mg^{2+} ($[Mg^{2+}]_i$) amounting to 0.25 mM [7, 8], the remaining part is bound to various ligands. Erythrocyte and cellular Mg^{2+} may result from Mg^{2+} influx and Mg^{2+} efflux, provided that the erythrocyte membrane is permeable to Mg^{2+}. With ^{28}Mg a small uptake of radioactive Mg^{2+} was found. However, only a part of erythrocyte Mg^{2+} was exchangeable [9, 10]. The reason for the limited Mg^{2+} exchange is not known. If extra- and intraerythrocyte Mg^{2+} were in equilibrium, Mg^{2+} should be steadily transported out of the cell, because the electrochemical gradient would drive Mg^{2+} into the cell.

However, so far a significant net Mg^{2+} efflux from erythrocytes was only measured when the cells had been loaded with Mg^{2+} [11–13]. In human and rat erythrocytes, a Na^+ and ATP-dependent Mg^{2+} efflux via Na^+/Mg^{2+} antiport and a Na^+- and ATP-independent Mg^{2+} efflux accompanied by Cl^- efflux for charge compensation were found [11, 12].

Na^+/Mg^{2+} antiport and Na^+-independent Mg^{2+} efflux had different transport capacities in human, rat and chicken

erythrocytes [14]. In Mg^{2+}-loaded rat erythrocytes Na^+/Mg^{2+} antiport was reversible when the extra-/intracellular Na^+ gradient was reversed [15], whereas in human erythrocytes Na^+/Mg^{2+} antiport seemed to be irreversible [16].

Another function of Na^+/Mg^{2+} antiport is the transport of Mn^{2+} instead of Mg^{2+}. Rat erythrocytes could take up Mn^{2+} via Na^+/Mg^{2+} antiport [17–19], whereas in chicken erythrocytes Mn^{2+} uptake is operating by Mn^{2+}/H^+ antiport [20].

These results indicate that there may be considerable differences in Mg^{2+} concentration and Mg^{2+} transport systems in erythrocytes of various species. Therefore, we investigated in erythrocytes of various species total and free Mg^{2+} concentration as well as Na^+/Mg^{2+} antiport, Na^+ independent Mg^{2+} efflux, reversal of Na^+/Mg^{2+} antiport and Mn^{2+} uptake.

Materials and methods

Blood was taken by heart puncture from anaesthetized rats, guinea pigs and mice (50 mg/kg Nembutal i.p.) and by venous puncture from human, pig, horse, sheep, cattle, cat and dog by means of a heparinized syringe. Blood from rabbit, duck, trout and chicken was sampled in heparin containing vessels during commercial slaughtering. 25 µl heparin solution (5000 U/ml, Sigma) was added per 5 ml blood. Blood was centrifuged at 1000 g for 5 min. Plasma and buffy coat were aspirated and red cells washed twice with 150 mM NaCl.

Mg^{2+}-loading

The cells were loaded with Mg^{2+} by incubating a 10 % cell suspension for 30 min at 37 °C (trout at 17 °C) in KCl medium (in mM: 140 KCl, 50 sucrose, 5 glucose, 30 Hepes/Tris, pH 7.4) with the addition of 12 mM $MgCl_2$ and ionophore A23187 (dissolved in dimethyl sulfoxide), the medium was slightly hyperosmotic to prevent hemolysis. The concentration of A23187 amounted to 6 and 12 µM for erythrocytes from cattle, to 3 and 6 µM for erythrocytes from horse and dog and to 6 µM for erythrocytes of the other species. For removal of the ionophore, the cells were incubated 4 times in ionophore free loading medium with the addition of 1 % bovine serum albumin (Sigma) for 10 min at 37 °C or 17 °C (trout).

Mg^{2+} and Mn^{2+} transport

Mg^{2+} efflux was measured by reincubation of a 10 % cell suspension at 37 °C (trout at 17 °C) in NaCl or choline Cl medium. NaCl or choline Cl medium was prepared by substitution of KCl in KCl medium by 140 mM NaCl or 140 mM choline Cl. At the beginning of reincubation and after 20, 30 or 40 min, 0.5 ml aliquots of the cell suspensions were centrifuged for 1 min at 10,000 g. For Mg^{2+} determination, 100 µl supernatant was diluted with 1 ml 10 % trichloroacetic acid (TCA)/ 0.175 % $LaCl_3$, and Mg^{2+} was measured by atomic absorption spectrophotometry (AAS, Philips SP9). For measuring Mn^{2+}-induced Mg^{2+} efflux, $MnCl_2$ was added to the reincubation media as indicated. Mn^{2+} influx was determined from the reduction of Mn^{2+} concentration in the reincubation media by means of AAS.

For measuring the reversal of Na^+/Mg^{2+} antiport the cells were additionally loaded with Na^+ by incubating the 10 % cell suspension in NaCl medium with 1 mM $MgCl_2$ in the presence of 6 µM A23187 and 30 µg/ml nystatin (Sigma) for 30 min at 37 °C. The NaCl concentrations amounted to 0, 10, 30, 50, 75 and 150 mM, isoosmotically substituted by choline Cl. For removal of A23187 and nystatin the cells were incubated 4 times in NaCl medium with the same NaCl and $MgCl_2$ as in the loading medium plus 1 % bovine serum albumin. For measuring Mg^{2+} transport (either efflux or influx) a 10% cell suspension was incubated in NaCl medium with different NaCl concentrations (0, 10, 30, 50, 75, 150 mM, isoosmotically substituted by choline Cl) and 0.5 mM $MgCl_2$ at 37 °C for 30 min.

The rates of Mg^{2+} flux were determined from the alteration of Mg^{2+} concentration in the reincubation media and were related to the cell volume measured by hematocrit.

Cellular Mg^{2+}, Na^+ and K^+ contents

For measuring cellular Mg^{2+}, Na^+ and K^+ contents, the cells were washed twice with 150 mM choline Cl and deproteinized with 5 % TCA. Mg^{2+} contents of the supernatants were measured by AAS, Na^+ and K^+ contents by flame photometry (KLINA FLAME, Beckman).

Intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$)

$[Mg^{2+}]_i$ was determined in erythrocytes from pig, rat and cow by ^{31}P-NMR. Heparinized blood was taken directly without the addition of D_2O to establish physiological conditions. ^{31}P-NMR measurements were performed on a Bruker AMX500 spectrometer with a ^{31}P-resonance of 202 MHz, equipped with a 10 mm multinuclear probe head. The probe head temperature was held constant at 310° K. The resonance frequency of a reference sample (phosphoric acid) was determined at the beginning and at the end of each measurement. For all spectra 64 K time domain data points were recorded using a 90° pulse, a relaxation delay of 1 s and a sweep width of 125 kHz. Proton decoupling was applied. For rat and pig blood, 1 K acquisition and for bovine erythrocytes 4 K scans were averaged because of their low ATP [1]. Line broadening of 12 Hz and manual baseline correction were applied to the spectra. The chemical shifts of the ATP signals were determined by means of a Lorentzian deconvolution (WINNMR algorithm).

Free Mg^{2+} concentration ($[Mg^{2+}]_i$) in erythrocytes was calculated according to Equation (1) [21].

$[Mg^{2+}]_i = K_D^{MgATP} \times (\phi^{-1} - 1)$ (1)
For K_D^{MgATP} (dissociation constant of MgATP) 50 µM was taken [21].
ϕ was calculated from the chemical shift differences of the α- and β-phosphoryl group resonance according to Eq. (2)
$\phi = (\delta_{\alpha\beta}^{x} - \delta_{\alpha\beta}^{MgATP}) / (\delta_{\alpha\beta}^{ATP} - \delta_{\alpha\beta}^{MgATP})$ (2)
Values for $\delta_{\alpha\beta}^{x}$, $\delta_{\alpha\beta}^{ATP}$ and $\delta_{\alpha\beta}^{MgATP}$ were taken from the spectra from blood, ATP and MgATP solutions (Mg^{2+} excess), respectively [22].

Statistics
The correlation coefficients were calculated by using Graph Pad Prism.

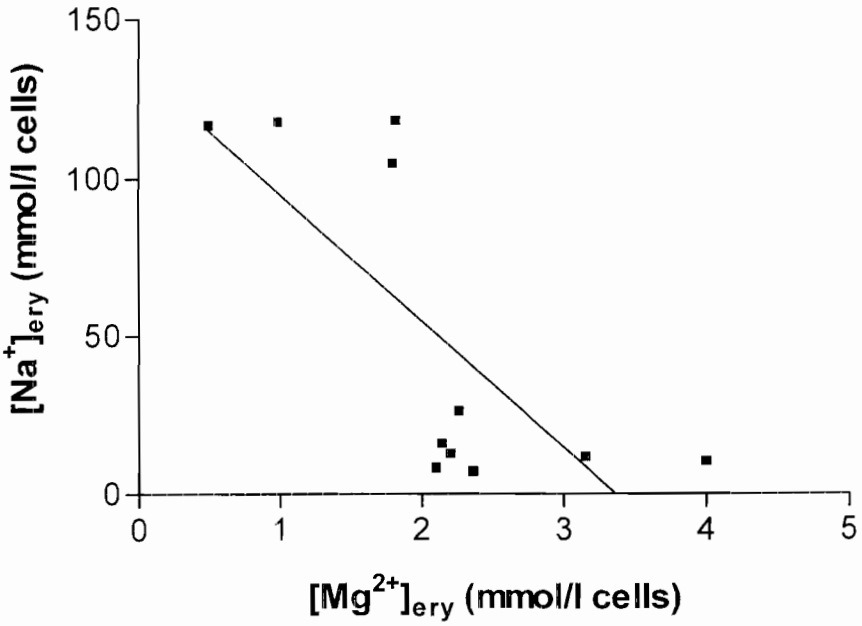

Fig. 1: Negative correlation between Na^+ and total Mg^{2+} concentrations of non-nucleated erythrocytes from various species. Values taken from table 1. **r = – 0.7331; p = 0.01**

Results and discussion

Mg^{2+} concentration in erythrocytes of various species

Values of total Mg^{2+} concentration of erythrocytes from various species are listed in table 1. The table also contains data of some previous publications. Since there are innumerous reports on erythrocyte Mg^{2+} content, for better comparison, only those values were included where the authors have simultaneously measured or reported total erythrocytes from various species. Comparing the values of these papers (table 1) very different values of total Mg^{2+} are obtained for the same species and also different relations among various species. These differences may be caused by methodological and genetical reasons. However, there are some rough rules.

1. Nucleated erythrocytes display a higher Mg^{2+} content than erythrocytes without a nucleus and other organelles such as mitochondria and microsomes. This can be explained by the high Mg^{2+} content of these organelles [23].

2. Erythrocytes from ruminants with a high Na^+ and low K^+ content express a low Mg^{2+} content, followed by carnivores. When calculating the correlation, a significant negative correlation of Mg^{2+} with Na^+ content (fig. 1) and

Tab. 1: Na^+, K^+, total Mg^{2+} ($[Mg]_t$) and free Mg^{2+} ($[Mg^{2+}]_f$) concentration of erythrocytes from various species. Mean ± SEM of 6 animals from each species. For comparison total Mg contents from other authors were also given.

	Na^+ mmol/l cells	K^+ mmol/l cells	$[Mg]_t$ mmol/l cells	$[Mg^{2+}]_f$ mM	$[Mg]_t$[a] mmol/kg w. w.	$[Mg]_t$[b] mmol/kg w. w.	$[Mg]_t$[c] mmol/l cells	$[Mg]_t$[d] mM
Man	15.9 ± 0.6	95.1 ± 4.2	2.14 ± 0.09	–	–	–	2.55	3.78
Rat	8.3 ± 0.3	127.0 ± 6.2	2.10 ± 0.10	0.22 ± 0.01	–	3.56	2.22	5.23
Mouse	12.8 ± 3.2	128.8 ± 11.6	2.20 ± 0.06	–	–	5.97	–	–
Rabbit	11.8 ± 0.7	125.2 ± 3.0	3.15 ± 0.14	–	1.89	3.87	3.74	6.22
Guinea pig	7.2 ± 0.4	122.5 ± 6.4	2.36 ± 0.06	–	–	4.03	3.2	–
Horse	26.3 ± 2.8	73.4 ± 4.4	2.26 ± 0.10	–	2.21	2.81	3.19	5.25
Pig	10.4 ± 2.1	90.0 ± 3.0	4.00 ± 0.18	0.22 ± 0.01	3.70	4.31	5.79	2.13
Sheep	118.0 ± 14.6	15.4 ± 0.7	0.99 ± 0.05	–	0.42	1.56	–	2.77
Cattle	117.0 ± 17.6	15.5 ± 1.1	0.50 ± 0.04	0.20 ± 0.01	0.43	0.74	1.18	3.18
Dog	118.5 ± 4.8	7.6 ± 0.3	1.82 ± 0.09	–	1.68	–	2.99	3.88
Cat	105.0 ± 6.0	3.9 ± 0.6	1.80 ± 0.05	–	2.00	–	2.80	–
Chicken	10.2 ± 1.6	96.2 ± 12.4	3.26 ± 0.11	–	–	3.80	–	10.28
Duck	6.4 ± 0.6	95.9 ± 2.1	3.85 ± 0.13	–	–	–	4.24	–
Trout	23.5 ± 1.8	78.5 ± 2.8	4.70 ± 0.39	–	–	–	–	–

a) calculated from [4]
b) calculated from [5]
c) mean of values calculated from [6]
d) taken from [1]

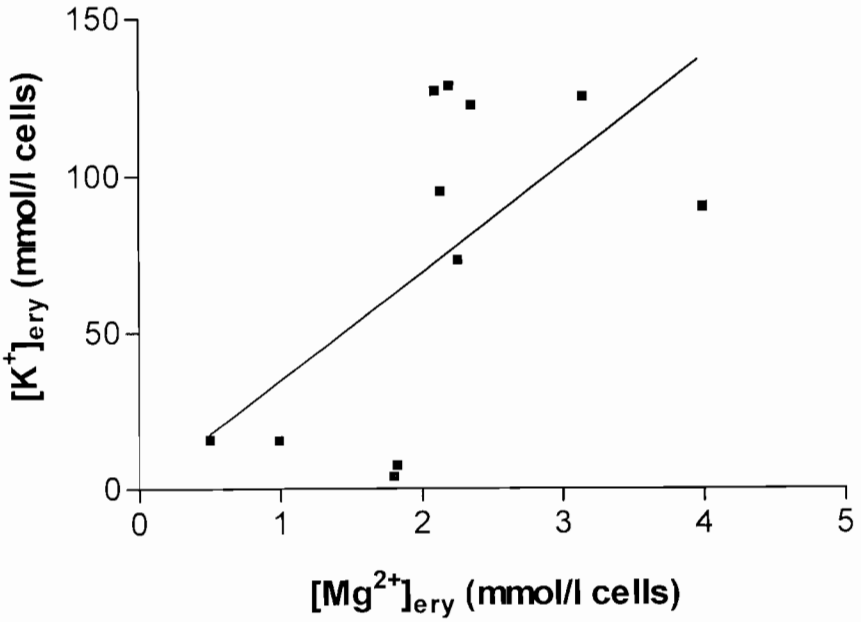

Fig. 2: Positive correlation between K^+ and total Mg^{2+} concentrations of non-nucleated erythrocytes from various species. Values taken from table 1. **r = 0.6120; p = 0.04**

a positive correlation of Mg^{2+} with K^+ (fig. 2) in non-nucleated erythrocytes are obtained. This may be explained by the different ATP content of erythrocytes which correlates with their Mg^{2+} content [1]. Although total Mg^{2+} of erythrocytes among mammals can differ by the factor 8 (pig – cattle, table 1), the concentration of free Mg^{2+} as measured by ^{31}P-NMR was the same. The value of 0.22 or 0.20 mM is in good agreement with the value of 0.25 mM measured in human erythrocytes by other authors [7, 8].

This result shows that Mg^{2+} dependent reactions are working under the same conditions in erythrocytes of various species with different total Mg^{2+}. Therefore, in erythrocytes of various species there must be a very different amount of bound Mg^{2+}. In fresh human red cells, 13 % of total Mg^{2+} was free Mg^{2+}, 34 % was bound to ATP, 18 % was bound to 2,3 bisphosphoglycerate (BPG) and 36 % was bound to an unidentified substance x [24]. When considering the Mg^{2+} complex binding substances ATP and 2,3 BPG, there is a weak correlation between Mg^{2+} and ATP [1] and between Mg^{2+} and 2,3 BPG [25] (fig. 3). However, these correlations can only give a preliminary explanation. A complete balance of bound Mg^{2+} is needed and the "unknown Mg^{2+} chelator" in erythrocytes [24] must be identified.

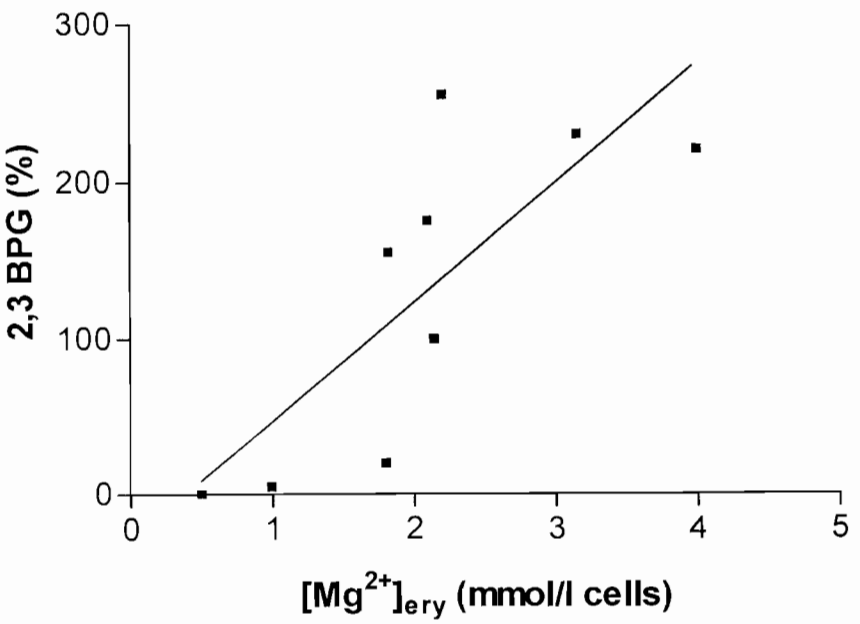

Fig. 3: Positive correlation between total Mg^{2+} content and concentration of 2,3 bisphosphoglycerate (2,3 BPG) in non-nucleated erythrocytes from various species. Total Mg^{2+} contents were taken from table 1. 2,3 BPG values were taken from [25]. 2,3 BPG is given in %, 100 % = 2,3 BPG in human erythrocytes. **r = 0.7740; p = 0.015**

Mg^{2+} efflux from erythrocytes of various species

Steady-state intracellular ion concentrations are usually explained by pump and leak. However, the pump leak theory is difficult to apply to mature erythrocytes. Mature erythrocytes only take up a small amount of Mg^{2+} when incubated at very high Mg^{2+} concentrations for long periods and when incubated in Mg^{2+} free medium, mature erythrocytes hardly loose any Mg^{2+} at all. When radioactive ^{28}Mg was injected to various species there was a very slow Mg^{2+} exchange in erythrocytes as compared to other cell types such as myocardium, liver and kidney. Mg^{2+} exchange in erythrocytes is operating with a fast component which amounted to 20 % of total Mg^{2+} after 2 hours and a slow component which reached 45–60 % of total Mg^{2+} after 7 hours [9, 10]. To explain the low rate of ^{28}Mg uptake, it was suggested that ^{28}Mg enters red cells only during erythropoiesis [26]. However, this suggestion disagrees with the low ^{28}Mg uptake of erythrocytes found in vitro [9].

In order to overcome these problems and to study Mg^{2+} transport in erythrocytes, we loaded erythrocytes artificially with Mg^{2+}. Mg^{2+}-loaded erythrocytes express Mg^{2+} efflux as

Tab. 2: Na^+/Mg^{2+} antiport and Na^+-independent Mg^{2+} efflux of erythrocytes from various species. Mean ± SEM of 6 animals from each species.

	Mg^{2+} content after loading mmol/l cells	Na^+/Mg^{2+} antiport mmol/l cells x 60'	Na^+-independent Mg^{2+} efflux mmol/l cells x 60'	Inhibition of total Mg^{2+} efflux by 1 mM amiloride %
Man	15.50 ± 0.80	0.38 ± 0.07	0.23 ± 0.03	74
Rat	15.90 ± 1.07	25.00 ± 1.99	1.97 ± 0.15	61
Mouse	24.82 ± 0.61	29.83 ± 1.68	2.13 ± 0.28	–
Rabbit	17.80 ± 1.13	27.88 ± 1.10	0.73 ± 0.10	90
Guinea pig	20.85 ± 0.34	33.10 ± 1.48	2.34 ± 0.18	61
Horse	21.87 ± 0.60	2.36 ± 0.22	0.94 ± 0.31	34
Pig	19.66 ± 1.23	7.60 ± 0.40	0.20 ± 0.04	91
Sheep	6.72 ± 0.65	0.75 ± 0.14	0.03 ± 0.01	30
Cattle	5.82 ± 0.64	0.12 ± 0.03	0.07 ± 0.02	44
Dog	27.20 ± 4.93	8.36 ± 1.63	11.07 ± 2.09	68
Cat	18.08 ± 0.85	7.65 ± 0.73	2.06 ± 0.51	41
Chicken	18.65 ± 1.87	4.20 ± 0.54	3.00 ± 0.98	66
Duck	16.57 ± 1.00	24.26 ± 1.64	1.15 ± 0.14	87
Trout	24.32 ± 1.09	18.82 ± 1.23	9.74 ± 1.30	68

long as the normal Mg^{2+} content is achieved [11, 12]. By this method, Na^+/Mg^{2+} antiport and Na^+-independent Mg^{2+} efflux were characterized. Table 2 shows that both components of Mg^{2+} efflux are very differently expressed in erythrocytes from various species. Rodents express high rates of Na^+/Mg^{2+} antiport and Na^+-independent Mg^{2+} efflux, whereas in ruminants both components of Mg^{2+} efflux are very low. In all species, Mg^{2+} efflux was inhibited by amiloride to different degrees (table 2).

The lower intracellular Mg^{2+} concentration of Mg^{2+}-loaded ruminant erythrocytes (table 2) is only partly responsible for their low rates of Na^+/Mg^{2+} antiport and Na^+-independent Mg^{2+} efflux. The rate of Na^+/Mg^{2+} antiport is dependent on the intracellular Mg^{2+} concentration. K_m for $[Mg^{2+}]_i$ at half maximal rate amounted to 3.5 mM for chicken, 3–4 mM for hamster and 1.3 mM or 2.6 mM for human erythrocytes [11]. K_m for $[Mg^{2+}]_i$ of Mg^{2+} efflux from ruminant erythrocytes was not estimated because of the very low transport rate, but may have a similar value as K_m for other erythrocytes. Thus, Mg^{2+} efflux from ruminant erythrocytes would be measured at conditions of more than half maximal velocity as can be seen from the intracellular Mg^{2+} concentration of Mg^{2+}-loaded erythrocytes (table 1).

All other rates of Na^+/Mg^{2+} antiport were measured at nearly V_{max} conditions. Therefore, the different rates of Na^+/Mg^{2+} antiport can be related to a different number of Na^+/Mg^{2+} antiporters. In the case of Na^+-independent Mg^{2+} efflux, the different rate may be caused by a different number of Mg^{2+} efflux channels.

Remarkably, erythrocytes from ruminants can be loaded to a lesser degree with Mg^{2+} under identical conditions than erythrocytes from other species.

Their total Mg^{2+} concentration was lower (6 mmol/l cells, table 2) than the Mg^{2+} concentration of the Mg^{2+} loading medium (12 mM). An explanation may be given by the result that divalent cation influx catalyzed by A23187 is decreased by a depolarization of the membrane potential [27]. Since erythrocytes from carnivores, which display a similarly low K^+_i/K^+_o gradient as erythrocytes from ruminants, are highly loaded with Mg^{2+} under the same conditions (table 2), other membrane effects may be additionally responsible for the different Mg^{2+}-loading. A different Mg^{2+} loading may be caused by a different Mg^{2+} binding capacity of the erythrocyte Mg^{2+} buffer. ATP can be excluded. ATP concentration in non-nucleated erythrocytes amounts only up to 1 mM [1] and at the physiological intracellular $[Mg^{2+}]_i$ the major part of ATP is already bound to Mg^{2+}. However, 2,3 BPG, its concentration amounting up to 20 mmol/l cells [28], may contribute to Mg^{2+} binding in some species during Mg^{2+} loading. Even though there are exceptions e.g. cat erythrocytes are highly loaded with Mg^{2+} (table 2) although their 2,3 BPG concentration is as low (1.5 mmol/l cells) as in cow and sheep [28].

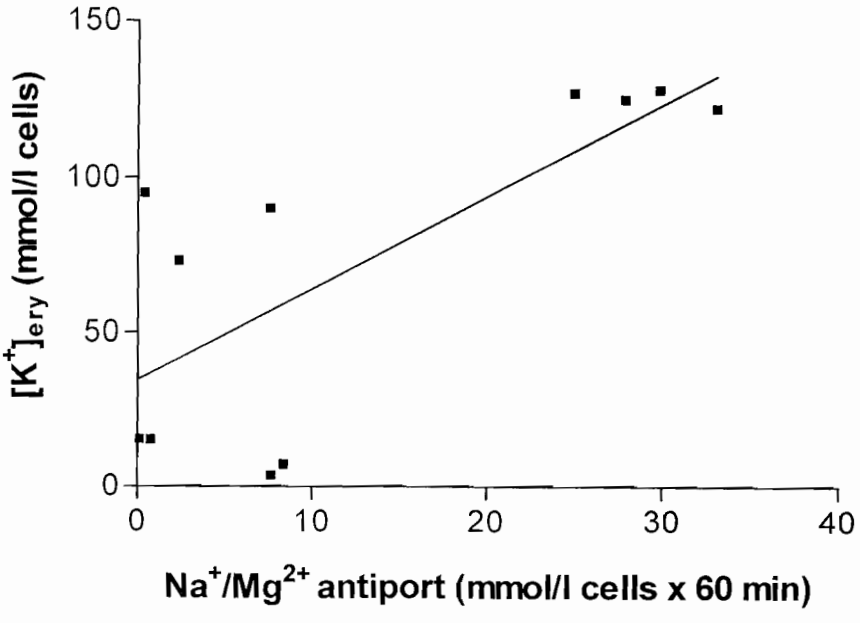

Fig. 4: Positive correlation between Na^+/Mg^{2+} antiport and K^+ concentration of non-nucleated erythrocytes from various species. Values taken from tables 1 and 2. **r = 0.7380; p = 0.009**

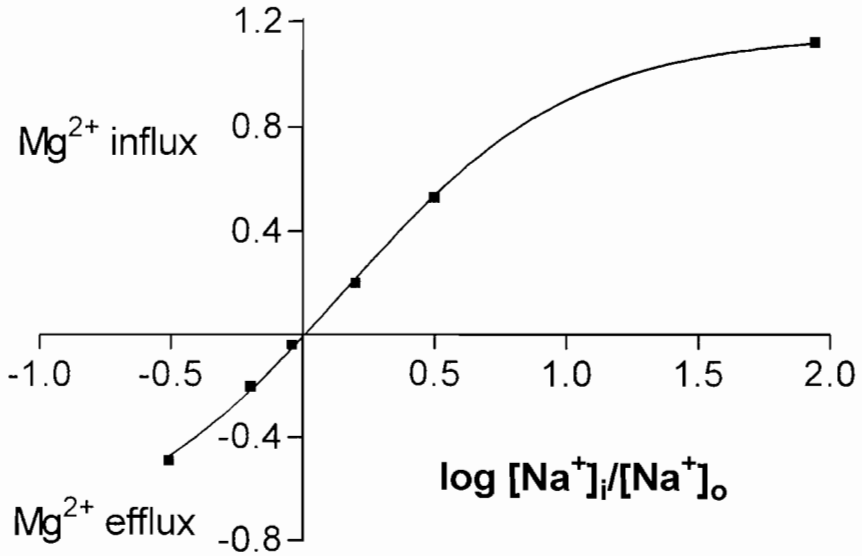

Fig. 5: Na^+/Mg^{2+} antiport (mmol/l cells x 30 min) in pig erythrocytes as a function of log Na^+_i/Na^-_o. At log $[Na^+]_i/[Na^-]_o > 0$ there is Mg^{2+} influx via Na^-/Mg^{2+} antiport. At log $[Na^+]_i/[Na^+]_o < 0$ (negative values) there is Mg^{2+} efflux via Na^+/Mg^{2+} antiport. Mean of 2 experiments.

Comparing the rates of Na^+/Mg^{2+} antiport and Na^+-independent Mg^{2+} efflux with other parameters of erythrocytes did not yield any correlation with Na^+, K^+ and Mg^{2+} contents in nucleated erythrocytes.

In non-nucleated erythrocytes, there was no significant correlation of Na^+-independent Mg^{2+} efflux with Na^-, K^+ and Mg^{2+} contents either (not shown). However, in non-nucleated erythrocytes, Na^+/Mg^{2+} antiport was significantly correlated with K^+ content. (fig. 4)

Na^+/Mg^{2+} antiport was not correlated with total Mg^{2+} concentration of erythrocytes with physiological Mg^{2+} levels from various species (not shown). From these results it may be concluded that Na^+/Mg^{2+} antiport is not involved when the different Mg^{2+} contents are established during formation and maturation of erythrocytes. However, there may be a rough correlation of Na^+/Mg^{2+} antiport with the rate of ^{28}Mg exchange. Erythrocytes from cat, dog and rat display a similar rate of Mg^{2+} exchange. Mg^{2+} exchange in human erythrocytes was about 10 times slower than in cat, dog and rat; and in bovine erythrocytes, Mg^{2+} exchange was even slower than in human erythrocytes and could not reliably be measured [9].

These results are in agreement with the model of Mg^{2+} transport in erythrocytes [13]. According to this model, in erythrocytes with normal Mg^{2+} content there is an Mg^{2+}-Mg^{2+} exchanger which is transformed to the Na^+/Mg^{2+} antiporter by loading the cells with Mg^{2+}. Hence, the number of Mg^{2+}/Mg^{2+} exchangers, which perform $^{28}Mg^{2+}/^{24}Mg^{2+}$ exchange, corresponds to the number of Na^+/Mg^{2+} antiporters.

Reversal of Na^+/Mg^{2+} antiport in erythrocytes of various species

Na^+/Mg^{2+} antiport was found to be reversed in rat erythrocytes [15]. Mg^{2+} efflux could be switched to Mg^{2+} influx via Na^+/Mg^{2+} antiport in Mg^{2+}-loaded erythrocytes when the Na^+_i/Na^+_o gra-

Tab. 3: Mn^{2+} uptake and Mn^{2+}-induced Mg^{2+} efflux from normal and Mg^{2+}-loaded erythrocytes (mmol/l cells x 20 min) at various extracellular Mn^{2+} concentrations ($[Mn^{2+}]_o$) in choline Cl medium ($-Na^+$) and NaCl medium ($+Na^+$). Mean ± SEM of 5 animals from each species.

	$[Mn^{2+}]_o$ mM	Mg^{2+}-efflux, normal Mg^{2+} $-Na^+$	Mg^{2+}-efflux normal Mg^{2+} $^+Na^+$	Mg^{2+}-efflux Mg^{2+}-loaded $-Na^+$	Mg^{2+}-efflux Mg^{2+}-loaded $^+Na^+$	Mn^{2+} uptake normal Mg^{2+} $-Na^+$	Mn^{2+} uptake normal Mg^{2+} $^+Na^+$	Mn^{2+} uptake Mg^{2+}-loaded $-Na^+$	Mn^{2+} uptake Mg^{2+}-loaded $^+Na^+$
Pig	0.25	–	–	0.33 ± 0.04	2.50 ± 0.42	–	–	0.25 ± 0.02	0.07 ± 0.01
	0.5	0.042 ± 0.006	0.066 ± 0.010	0.41 ± 0.03	2.41 ± 0.43	0.060 ± 0.011	0.017 ± 0.005	0.34 ± 0.02	0.14 ± 0.01
	1.0	0.057 ± 0.008	0.036 ± 0.012	0.41 ± 0.03	2.17 ± 0.39	0.066 ± 0.011	0.024 ± 0.007	0.41 ± 0.02	0.23 ± 0.01
	3.0	0.052 ± 0.014	0.044 ± 0.008	–	–	0.070 ± 0.007	0.034 ± 0.008	–	–
Cattle	0.25	–	–	0.016 ± 0.002	0.076 ± 0.010	0.0134 ± 0.0001	0.013 ± 0.001	0.038 ± 0.002	0.007 ± 0.010
	0.5	0.017 ± 0.003	0.011 ± 0.003	0.038 ± 0.003	0.061 ± 0.005	0.014 ± 0.001	0.017 ± 0.003	0.062 ± 0.003	0.014 ± 0.005
	1.0	0.018 ± 0.003	0.015 ± 0.010	0.023 ± 0.008	0.052 ± 0.008	0.033 ± 0.009	0.023 ± 0.005	0.053 ± 0.008	0.017 ± 0.006
	3.0	0.021 ± 0.001	0.019 ± 0.004						
Rat	0.25	–	–	1.07 ± 0.14	6.71 ± 0.58	–	–	0.51 ± 0.08	0.33 ± 0.03
	0.5	0.04 ± 0.01	0.06 ± 0.01	0.90 ± 0.31	5.81 ± 0.20	0.09 ± 0.02	0.07 ± 0.01	0.61 ± 0.08	0.53 ± 0.03
	1.0	0.04 ± 0.01	0.06 ± 0.02	–	–	0.10 ± 0.01	0.09 ± 0.02	–	–
	3.0	0.05 ± 0.02	0.03 ± 0.02	–	–	0.11 ± 0.02	0.11 ± 0.03	–	–
Chicken	0.25	–	–	0.66 ± 0.24	2.02 ± 0.23	–	–	0.51 ± 0.13	0.64 ± 0.07
	0.5	0.010 ± 0.000	0.010 ± 0.005	0.60 ± 0.18	1.72 ± 0.23	0.027 ± 0.005	0.023 ± 0.003	1.03 ± 0.09	1.03 ± 1.10
	1.0	0.017 ± 0.003	0.012 ± 0.004	0.67 ± 0.14	1.59 ± 0.24	0.047 ± 0.014	0.037 ± 0.010	1.84 ± 0.35	1.87 ± 0.23
	3.0	0.013 ± 0.005	0.010 ± 0.005	–	–	0.100 ± 0.029	0.090 ± 0.019	–	–

dient was reversed by additional Na^+-loading by means of nystatin and incubation in media with different Na^+ concentration. At $Na^+_i/Na^+_o = 1$ there was no Mg^{2+} flux, indicating that the Na^+ gradient under these conditions of low Mg^{2+} loading was the major driving force [15]. Reversal of Na^+/Mg^{2+} antiport was also found with Mg^{2+}-unloaded ferret erythrocytes [29], whereas in Mg^{2+}-loaded human erythrocytes Na^+/Mg^{2+} antiport seemed to be irreversible [16]. Therefore, to additionally characterize Na^+/Mg^{2+} antiporters, we tested the reversal of Mg^{2+} flux in Mg^{2+}-loaded pig erythrocytes, which possess a medium Na^+/Mg^{2+} antiport capacity compared to rat (high) and human (low) or cattle (low).

As shown in fig. 5, when Mg^{2+} and Na^+ loaded erythrocytes were incubated in Na^+ medium with $Na^+_i/Na^+_o < 1$ (negative log Na^+_i/Na^+_o) Mg^{2+} efflux was observed. At $Na^+_i/Na^+_o = 1$ (log $Na^+_i/Na^+_o = 0$) there was no Mg^{2+} flux and at $Na^+_i/Na^+_o > 1$ (log $Na^+_i/Na^+_o > 0$) Mg^{2+} was taken up. These results indicate that Na^+/Mg^{2+} antiport in pig erythrocytes is reversed and driven by the Na^+_i/Na^-_o gradient. The same result was found for rat erythrocytes [15].

Preliminary experiments with chicken erythrocytes (unpublished) revealed that Mg^{2-} transport in these cells was not reversed under the same experimental conditions. These results indicate that the Na^+/Mg^{2-} antiporter in chicken erythrocytes differs from that of non-nucleated erythrocytes.

Mn^{2+} uptake by erythrocytes from various species

Erythrocytes from rat [17, 18] and rabbit [19] can take up Mn^{2+} via the Na^-/Mg^{2+} antiporter, whereas human erythrocytes did not significantly take up Mn^{2+} [20]. In chicken erythrocytes, Mn^{2+} is transported by Mn^{2+}/H^+ antiport [20].

In order to extend these studies, we investigated Mg^{2+} efflux and Mn^{2+} influx in erythrocytes from pig and cow which, besides rat and rabbit, express a very different rate of Na^+/Mg^{2+} antiport. For comparison with previous results [20] erythrocytes from rat and chicken were included (table 3).

In not Mg^{2+}-loaded erythrocytes of all tested species, the addition of Mn^{2+} to the medium only caused a very small rate of Mg^{2+} efflux and Mn^{2+} influx. The values for Mg^{2+} efflux are almost the same as for Mn^{2+} influx. The values are not significantly different in media with Na^+ and without Na^+. These results indicate that in cells with normal Mg^{2+}

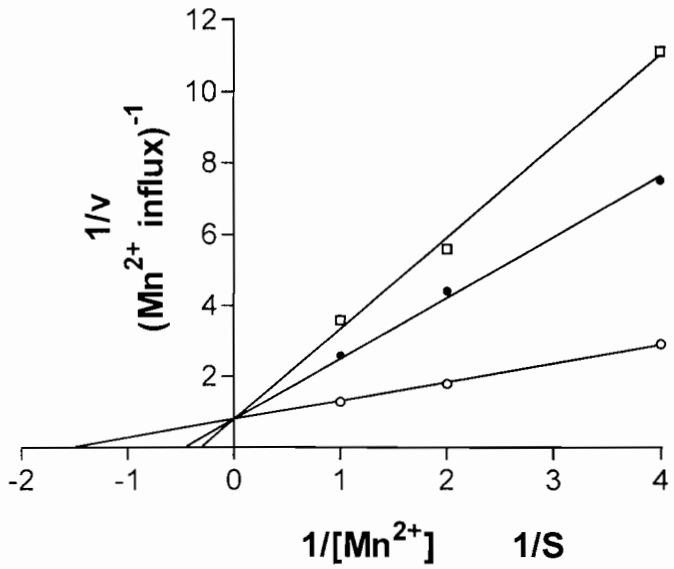

Fig. 6: Lineweaver-Burk plot of Mn^{2+} influx in Mg^{2+}-loaded pig erythrocytes in choline Cl medium (○, K_m= 0.65 mM), competitive inhibition of Mn^{2+} influx by extracellular Na^+(NaCl medium, ●, K_i= 61 mM).Competitive inhibition by 0.2 mM amiloride in NaCl medium (❑).
$\frac{1}{v}$ is given in (mmol/l cells x 40 min)$^{-1}$, $\frac{1}{S}$ in mM^{-1}.
Mean of 2 experiments.

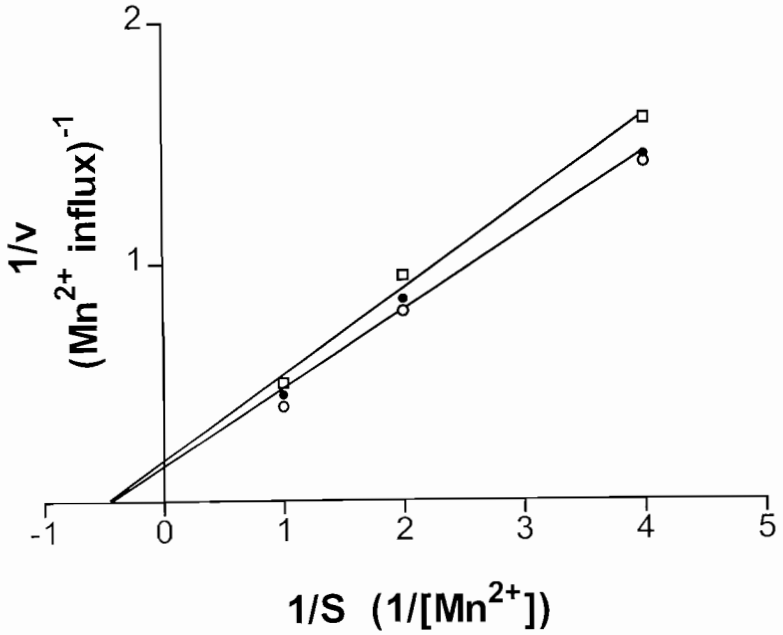

Fig. 7: Lineweaver-Burk plot of Mn^{2+} influx in Mg^{2+}-loaded chicken erythrocytes in choline Cl medium (○, K_m= 2.2 mM), no inhibition by extracellular Na^+(NaCl medium, ●), no significant inhibition by 1 mM amiloride in NaCl medium (❑).
$\frac{1}{v}$ is given in (mmol/l cells x 40 min)$^{-1}$, $\frac{1}{S}$ in mM^{-1}.
Mean of 2 experiments.

content, there is only a small rate of Mn^{2+}/Mg^{2+} exchange. The small rates of Mg^{2+} efflux and Mn^{2+} influx are inhibited by 1 mM amiloride, further indicating that Mg^{2+} efflux and Mn^{2+} influx in erythrocytes is operating via the Mg^{2+}/Mg^{2+} exchanger [13].

When erythrocytes are loaded with Mg^{2+}, the Mg^{2+}/Mg^{2+} exchanger in rat and pig erythrocytes may be transformed to the Na^+/Mg^{2+} antiporter [13] that transports Mg^{2+} and Mn^{2+} at a higher rate. In Na^+-free medium the ratios of Mg^{2+} efflux and Mn^{2+} influx in pig, cow and rat erythrocytes at various extracellular Mn^{2+} are nearly 1 : 1, indicating Mn^{2+}/Mg^{2+} exchange. When Na^+ is present in the medium, there is an additional Na^+/Mg^{2+} antiport increasing Mg^{2+} efflux. At the same time, Mn^{2+} influx via Na^+/Mg^{2+} antiport is much less than in Na^+ free media. This result indicates that under these conditions extracellular Na^+ and Mn^{2+} are competing in Na^+/Mg^{2+} antiport resulting in a simultaneous Mn^{2+}/Mg^{2+} antiport. These results are more clearly demonstrated by Lineweaver Burk plot (fig. 6). In pig erythrocytes, K_m for Mn^{2+} influx in Na^+-free medium (Mn^{2+}/Mg^{2+} antiport) amounted to 0.65 mM. Extracellular Na^+ inhibits competitively (K_i = 61 mM) and amiloride is also a competitive inhibitor. Mg^{2+}-loaded cow erythrocytes show a similar result, however, to a much lesser degree. This indicates that the Na^+/Mg^{2+} antiport in cow erythrocytes has the same properties. However, the number of Na^+/Mg^{2+} antiporters in cow erythrocytes is extremely low.

The behavior of Mn^{2+} influx in chicken erythrocytes differs from that of non-nucleated erythrocytes. Mn^{2+} influx in Mg^{2+}-loaded chicken erythrocytes occurs at lower affinity, K_m = 2.2 mM, Mn^{2+} influx is not inhibited by extracellular Na^+, and Mn^{2+} influx is only poorly inhibited by 1 mM amiloride (fig. 7), which strongly inhibits Na^+/Mg^{2+} antiport in nucleated erythrocytes (table 2).

These results indicate that Mn^{2+} influx in chicken erythrocytes is not operating via Na^+/Mg^{2+} antiport. In previous experiments it was found that Mn^{2+} influx functions via Mn^{2+}/H^+ antiport [20]. Therefore, it must be concluded that the Na^+/Mg^{2+} antiporter from chicken erythrocytes, although mediating electroneutral exchange of Na^+ for Mg^{2+}, is different from the Na^+/Mg^{2+} antiporter in pig erythrocytes. This also indicates that Na^+/Mg^{2+} antiporter of chicken erythrocytes expresses a higher specificity.

In conclusion, the Na^+/Mg^{2+} antiporter seems to belong to a specific family of transporters and may be related to Na^+/H antiport. In nucleated erythrocytes (chicken) this transport system is more specific than in non-nucleated ones.

References

[1] *Miseta, A.; Bogner, P.; Berenyi, E.; Kellermayer, M.; Galambos, C.; Wheatley, D.N.; Cameron, I.L.:* Relationship between cellular ATP, potassium, sodium and magnesium concentration in mammalian and avian erythrocytes. Biochim. Biophys. Acta 1175 (1993) 133-139.

[2] *Chan, P.C.; Calabrose, V.; Theil, L.S.:* Species differences in effect of sodium and potassium ions on ATPase of erythrocyte membrane. Biochim. Biophys. Acta 79 (1964) 424-426.

[3] *Maede, Y.; Inaba, M.:* (Na, K) ATPase and ouabain binding in reticulocytes from dogs with high K and low K erythrocytes and their changes during maturation. J. Biol. Chem. 260 (1985) 3337-3343.

[4] *Greenberg, D.M.; Lucia, S.P.; Mackey, M.A.; Tufts, E.V.:* The magnesium content of the plasma and the red corpuscles in human blood. J. Biol. Chem. 100 (1933) 139-148.

[5] *Eveleth, D.F.:* Comparison of the distribution of magnesium in blood cells and plasma of animals. J. Biol. Chem. 199 (1937) 289-292.

[6] *Walser, M.:* Magnesium metabolism. Rev. Physiol. Biochem. Exptl. Pharm. 59 (1967) 185-296.

[7] *Gupta, R.K.; Benovic, J.L.; Rose, Z.B.:* The determination of the free magnesium level in the human red blood cell by ^{31}PNMR. J. Biol. Chem. 254 (1978) 6172-6176.

[8] *Jelicks, L.A.; Weaver, J.; Pollack, S.; Gupta, R.K.:* NMR studies of intracellular free calcium, free magnesium and sodium in the guinea pig reticulocyte and mature red cell. Biochim. Biophys. Acta 1012 (1989) 261-266.

[9] *Rogers, T.A.:* The exchange of radioactive magnesium in erythrocytes of several species. J. Cell. Comp. Physiol. 57 (1961) 119-121.

[10] *Rogers, T.A.; Mahan, P.E.:* Exchange of radioactive magnesium in the rat. Proc. Soc. Exp. Biol. Med. 100 (1959) 235-239.

[11] *Günther, T.:* Mechanisms and regulation of Mg^{2+} efflux and Mg^{2-} influx. Miner. Electrolyte Metab. 19 (1993) 259-265.

[12] *Vormann, J.; Günther, T.:* Magnesium transport mechanisms. In: *Birch, N.J.* (ed) Magnesium and the Cell. Academic Press, (1993) pp 137-155.

[13] *Günther, T.:* Putative mechanism of Mg^{2+}/Mg^{2+} exchange and Na^-/Mg^{2-} antiport. Mg.-Bull. 18 (1996) 2-6.

[14] *Günther, T.; Vormann, J.:* Characterization of Mg^{2+} efflux from human, rat and chicken erythrocytes. FEBS Lett. 250 (1989) 633-637.

[15] *Günther, T.; Vormann, J.:* Reversibility of Na^+/Mg^{2+} antiport in rat erythrocytes. Biochim. Biophys. Acta 1234 (1995) 105-110.

[16] *Schatzmann, H.J.:* Asymmetry of the magnesium sodium exchange across the human red cell membrane. Biochim. Biophys. Acta 1148 (1993) 15-18.

[17] *Feray, J.C.; Garay, R.:* A onc-to-one Mg^{2+}: Mn^{2+} exchange in rat erythrocytes. J. Biol. Chem. 262 (1987) 5763- 5768.

[18] *Günther, T.; Vormann, J.; Cragoe Jr., E.J.:* Species-specific Mn^{2+}/Mg^{2+} antiport from Mg^{2+}-loaded erythrocytes. FEBS Lett. 261 (1990) 47-51.

[19] *Chua, A.C.G.; Stonell, L.M.; Savigni, D.L.; Morgan, E.H.:* Mechanisms of manganese transport in rabbit erythroid cells. J. Physiol. 493 (1996) 99-112.

[20] *Günther, T.; Vormann, J.:* Induction of Mn^{2+}/H^+ antiport in chicken erythrocytes by intracellular Mg^{2+} and Mn^{2+}. FEBS Lett. 265 (1990) 55-58.

[21] *London, R.E.:* Methods for measurement of intracellular magnesium: NMR and fluorescence. Ann. Rev. Physiol. 53 (1991) 231-258.

[22] *Günther, T.; Vormann, J.; Konstanczak, P.; Schäfer, A.:* Interactions of polyamines in the measurement of free magnesium concentration by mag-fura-2 and ^{31}P-NMR. Biochim. Biophys. Acta 1192 (1994) 281-285.

[23] *Ebel, H.; Günther, T.:* Magnesium metabolism. J. Clin. Chem. Clin. Biochem. 18 (1980) 257-270.

[24] *Bock, J.L.; Yusuf, Y.:* Further studies on alterations in magnesium binding during cold storage of erythrocytes. Biochim. Biophys. Acta 941 (1988) 225-231.

[25] *Torrance, J.D.:* Erythrocyte 2,3 –DPG in various mammalian species. In: *Gerlach, E.; Moser, K.; Deutsch, E.; Willmanns, W.* (eds) Erythrocytes, Thrombocytes, Leukocytes. Georg Thieme, Stuttgart (1973) pp 161-164.

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[26] *Watson, W.S.; Hilditch, T.E.; Horton, P.W.; Davies, D.L.; Lindsay, R.:* Magnesium metabolism in blood and the whole body in man using ^{28}magnesium. Metabolism 28 (1979) 90-95.

[27] *Fasolato, C.; Pozzan, T.:* Effect of membrane potential on divalent cation transport catalyzed by the "electroneutral" ionophores A23187 and ionomycin. J. Biol. Chem. 264 (1989) 19630-19636.

[28] *Bartlett, G.R.:* Patterns of phosphate compounds in red blood cells of man and animals. Adv. Exptl. Med. Biol. 6 (1969) 245-256.

[29] *Flatman, P.W.; Smith, L.M.:* Sodium-dependent magnesium uptake by ferret red cells. J. Physiol. 443 (1991) 217-230.

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