# Magnesium Concentrations in Serum and Erythrocytes of Ruminants, Perissodactyla and Primates in the Prague ZOO

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*Received December 20, 2004*
*Accepted June 3, 2005*

**Abstract**

Wilhelm Z., A. Pechová, R. Vodička: Magnesium Cncentration in Serum and Erythrocytes of Ruminants, Perissodactyla and Primates in the Prague ZOO. Acta Vet. Brno 2005, 74: 467-473.

The aim of the study was to assess the concentrations of magnesium in serum and erythrocytes of selected healthy animals, i.e., ruminants (R; n = 55), perissodactyla (P; n = 32) and primates (Pr; n = 7), kept in the Prague ZOO. Hematocrit values and serum magnesium concentrations were determined in venous blood samples. Magnesium concentrations were also assessed in full hemolysed blood, and the values of magnesium concentration in erythrocytes were calculated. Magnesium concentrations ($mmol \cdot l^{-1}$) found in serum (R: 0.83 ± 0.16; P: 0.65 ± 0.09; Pr: 0.81 ± 0.09) showed differences between the animal groups (R vs P, $p < 0.001$; P vs Pr $p < 0.001$; R vs Pr non-significant). Magnesium concentrations in erythrocytes (R: 1.25 ± 0.46; P: 2.35 ± 0.37; Pr: 1.51 ± 0.15) likewise showed significant differences when the groups were compared (R vs P, $p < 0.001$, P vs Pr, $p < 0.001$, R vs Pr, non-significant). Our results indicate that erythrocyte and serum concentrations of magnesium were not related. For the asessment of magnesium in tissues, its concentration in erythrocytes as a marker appears to be more reliable.

*Blood, animals, minerals*

With growing awareness of the role of magnesium in the organism, this ion has been monitored with increasing frequency also in veterinary medicine (Stewart et al. 2004). Over three hundred enzymatic reactions in the body are associated with the presence of magnesium, and further data on its role and importance appear every year. The information is based on studies involving both experimental animals (Wijnberg et al. 2002) and field observations (Tohno et al. 2001; Garcia-Lopez et al. 2001 Sevinga et al. 2002; Lopes et al. 2002; Johanson et al. 2003; Donovan et al. 2004).

Magnesium, as an intracellular cation, is involved in a number of biochemical reactions in the cell; for instance, its presence is essential for the production of adenosine triphosphate (ATP) molecules. It is therefore important to know the concentration of magnesium in tissues. One of the tissues easy to collect is blood, i.e., its anucleate cells – erythrocytes. Magnesium levels found in erythrocytes are, in contrast to serum levels, more stable, because they are less affected by single-dose intakes of magnesium (Wilhelm 2002; Wilhelm 2004).

Since the assessment of magnesium concentrations in erythrocytes is not included in routine examination of animals, we investigated this ion's presence in the framework of regular health check-ups carried out in the Prague ZOO.

The aim of the study is to ascertain whether magnesium concentrations differ between serum and erythrocytes and, if so, what their relationship is, and whether significantly different levels of magnesium in both serum and erythrocytes can be found in different genera (ruminants, perissodactyla, primates).

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### Materials and Methods

Ninety-four venous blood samples were collected from selected mammalian species during regular preventive veterinary check-ups in the Prague ZOO. No feed was available to the animals for 24 h before blood collection, but they had free access to drinking water. All animals were clinically healthy.

Three groups of animals were investigated: ruminants, perissodactyla and primates. The ruminant group (55) consisted of *Adax nasomaculatus* (n = 3), *Hippotragus niger* (n = 7), *Bison bison* (n = 2), *Cervus eldii* (n = 3), *Oreamnos americanus* (n = 3), *Capra domestica* (n = 2), *Capra caucasica* (n = 2), *Lama alpaca* (n =1), *Alces alces alceslos* (n = 4), *Elaphurus davidianus* (n = 1), *Tragelaphus angasii* (n = 3), *Boselaphus tragocamelus* (n = 2), *Oryx dammah* (n = 1), *Pseudois nayaur* (n = 2), *Ammotragus lervia* (n = 2), *Pudu pudu* (1), *Tragelaphus spekei* (n = 5), *Camelus ferus* (n = 6), *Cervus alaphus manitobensis* (n = 3), *Bison bonasus bonasus* (n = 1), *Giraffa camelopardalis rotschildi* (n = 1). The ruminant group also included camels, which have a similar digestive system, although they constitute an independent biological group. The perissodactyla comprised (n = 32) *Equus hemionus kulan* (n = 3), *Equus przewalskii* (n = 15), *Equus kiang* (n = 7), *Equus zebra hartmannae* (n = 7). The primates (n = 7) included *Gorilla gorilla gorilla* (n = 2), *Macaca sylvanus* (n = 1), *Pongo pygmaeus abelii* (n = 2), *Pan troglodytes* (n = 2). Because differences in ion concentrations between genders were not studied, each group included animals of both sexes.

Serum and erythrocytes were analyzed for the presence of magnesium as well as calcium as a natural magnesium-antagonist. The blood samples were collected into two disposable tubes, one with heparin for whole blood and the second without any anticoagulants for serum. Heparinized blood was used to determine the concentration of magnesium in erythrocytes, whole blood was hemolysed with distilled water (1 : 3). Serum was analyzed for magnesium and calcium and hemolysed blood was analyzed only for magnesium by flame atomic absorption spectrofotometry using an H 1550 (Hilger, Great Britain). Magnesium values in full hemolysed blood were obtained, and the magnesium concentration in erythrocytes was calculated as follows:

$$eryMg^{2+} = \frac{hemMg^{2+} - sMg^{2+}(1 - Ht)}{Ht}$$

$eryMg^{2+}$ - magnesium concentration in erythrocytes ($mmol \cdot l^{-1}$)
$hemMg^{2+}$ - magnesium concentration in full hemolysed blood ($mmol \cdot l^{-1}$)
$sMg^{2+}$ - magnesium concentration in serum ($mmol \cdot l^{-1}$)
Ht - hematocrit

All biochemical indices were assayed at biochemical laboratory of the Clinic of Diseases of Ruminants at University of Veterinary and Pharmaceutical Sciences Brno.

The results were evaluated using the Mann-Whitney's and Wilcoxon's rank test.

## Results

We found that the difference between serum and erythrocyte concentrations of magnesium was significant in every group. Magnesium concentrations in serum and erythrocytes and serum calcium levels are presented for the ruminants, perissodactyla and primates in Tables 1, 2 and 3, respectively.

The average values of magnesium in serum, magnesium in erythrocytes and calcium in serum for all groups are shown in Figs 1, 2 and 3, respectively.

These presentations clearly show that, on comparison of all three groups, there are differences in cation levels.

The differences in serum magnesium concentration among the three groups of animals are shown in Fig. 1. This comparison is also presented for magnesium concentrations in erythrocytes (Fig. 2) and calcium levels in serum (Fig. 3). The relationship between the erythrocyte and serum concentrations of magnesium for all three groups can be seen in Fig. 4. The values of erythrocyte concentration are not related to serum concentration values. The relationship between serum and erythrocyte concentrations is linear for each group, i.e., ruminants, perissodactyla and primates (Fig. 5).

## Discussion

Serum concentrations of magnesium can easily be affected by magnesium intake at the time of analysis. On the other hand, its erythrocyte concentrations are not influenced by administration or omission of a single dose because the magnesium level in an erythrocyte

Table 1. Magnesium and calcium levels in ruminants and camels (n = 55) $sMg^{2+}$, serum concentration of magnesium; $eryMg^{2+}$, concentration of magnesium in erythrocytes; $sCa^{2+}$, serum concentration of calcium. Values are presented as means ± SD.

| **Species** | **No. of animals** | | **Cation** (mmol·l$^{-1}$) | |
|---|---|---|---|---|
| | | **$sMg^{2+}$** | **ery $Mg^{2+}$** | **$sCa^{2+}$** |
| *Addax nasomaculatus* | 3 | 0.85 ± 0.17 | 1.23 ± 0.41 | 2.37 ± 0.09 |
| *Hippotragus niger* | 7 | 0.79 ± 0.08 | 0.87 ± 0.10 | 2.27 ± 0.14 |
| *Bison bison* | 2 | 0.80 ± 0.01 | 0.82 ± 0.04 | 2.30 ± 0.11 |
| *Cervus eldii* | 3 | 0.87 ± 0.06 | 1.01 ± 0.19 | 2.00 ± 0.17 |
| *Oreamnos americanus* | 3 | 0.91 ± 0.03 | 1.49 ± 0.12 | 2.40 ± 0.23 |
| *Capra domestica* | 2 | 0.75 ± 0.05 | 1.00 ± 0.22 | 2.16 ± 0.0 |
| *Capra caucasica* | 2 | 0.88 ± 0.08 | 1.01 ± 0.14 | 2.44 ± 0.35 |
| *Lama alpaca* | 1 | 0.86 | 1.86 | 2.49 |
| *Alces alces alces* | 4 | 0.69 ± 0.10 | 1.71 ± 0.31 | 2.41 ± 0.13 |
| *Elaphurus davidianus* | 1 | 0.70 | 0.85 | 2.42 |
| *Tragelaphus angasii* | 3 | 1.03 ± 0.08 | 1.21 ± 0.32 | 2.70 ± 0.05 |
| *Boselaphus tragocamelus* | 2 | 0.65 ± 0.04 | 1.36 ± 0.09 | 2.16 ± 0.23 |
| *Oryx dammah* | 1 | 0.77 | 0.58 | 2.00 |
| *Pseudois nayaur* | 2 | 0.85 ± 0.07 | 1.53 ± 0.08 | 2.36 ± 0.05 |
| *Ammotragus lervia* | 2 | 0.64 ± 0.04 | 1.12 ± 0.01 | 2.19 ± 0.12 |
| *Pudu pudu* | 1 | 1.53 | 1.36 | 1.71 |
| *Tragelaphus spekei* | 5 | 0.96 ± 0.09 | 0.96 ± 0.32 | 2.49 ± 0.17 |
| *Camelus ferus* | 6 | 0.80 ± 0.21 | 1.85 ± 0.61 | 2.45 ± 0.12 |
| *Cervus elaphus manitobensis* | 3 | 0.75 ± 0.03 | 1.12 ± 0.67 | 2.45 ± 0.20 |
| *Bison bonasus bonasus* | 1 | 0.70 | 1.05 | 2.26 |
| *Giraffa camelopardalis rotchildi* | 1 | 1.07 | 2.10 | 2.44 |

Table 2. Magnesium and calcium levels in perissodactyla (n = 32) $sMg^{2+}$, serum concentration of magnesium; $eryMg^{2+}$, concentration of magnesium in erythrocytes; $sCa^{2+}$, serum concentration of calcium. Values are presented as means ± SD.

| **Species** | **No. of animals** | **Cation** (mmol·l$^{-1}$) | | |
|---|---|---|---|---|
| | | **$sMg^{2+}$** | **ery $Mg^{2+}$** | **$sCa^{2+}$** |
| *Equus hemionus kulan* | 3 | 0.67 ± 0.04 | 1.65 ± 0.08 | 2.38 ± 0.07 |
| *Equus przewalskii* | 15 | 0.68 ± 0.11 | 2.35 ± 0.32 | 2.65 ± 0.24 |
| *Equus kiang* | 7 | 0.69 ± 0.06 | 2.39 ± 0.28 | 2.49 ± 0.24 |
| *Equus zebra hartmannae* | 7 | 0.56 ± 0.05 | 2.69 ± 0.24 | 2.34 ± 0.20 |

is based on that in the stem cell from which the particular erythrocyte will develop. Therefore the information provided by the serum value is different from that derived from the erythrocyte value. Serum concentrations reflect the present situation, while erythrocyte values show a long-term status (Sacks 1997). This is also related to the fact that only about 1% of erythrocytes is replaced daily and that intracellular concentrations of magnesium directly affect cell's physiological functions.

Table 3. Magnesium and calcium levels in primates (n = 7) $sMg^{2+}$, serum concentration of magnesium; $eryMg^{2+}$, concentration of magnesium in erythrocytes; $sCa^{2+}$, serum concentration of calcium. Values are presented as means ± SD.

| Species | No. of animals | Cation ($mmol \cdot l^{-1}$) | | |
|---|---|---|---|---|
| | | $sMg^{2+}$ | ery $Mg^{2+}$ | $sCa^{2+}$ |
| *Gorilla gorilla gorilla* | 2 | 0.77 ± 0.15 | 1.42 ± 0.06 | 2.55 ± 0.0 |
| *Macaca sylvanus* | 1 | 0.80 | 1.87 | 2.43 |
| *Pongo pygmaeus abelii* | 2 | 0.83 ± 0.11 | 1.54 ± 0.05 | 2.31 ± 0.06 |
| *Pan troglodytes* | 2 | 0.86 ± 0.12 | 1.40 ± 0.07 | 2.18 ± 0.11 |

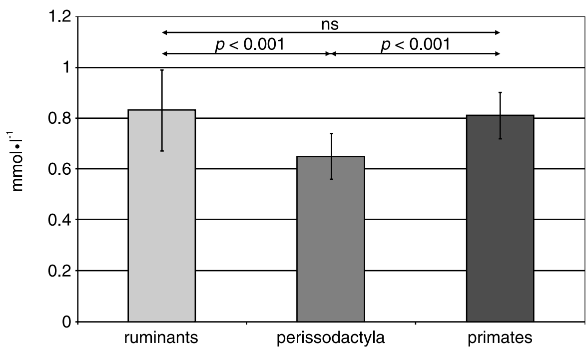


Fig. 1. Magnesium concentrations in sera of the animal groups

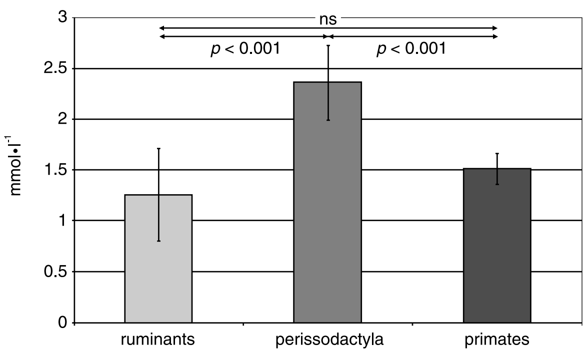


Fig. 2. Magnesium concentrations in erythrocytes of the animal groups

Magnesium concentrations in serum were highest in the ruminants, while its concentration in erythrocytes was lowest. The serum concentrations of calcium were lower in the ruminants than in perissodactyla. The increased concentrations of magnesium in erythrocytes in the perissodactyla group can indicate a sufficient saturation of tissues with this cation, and may suggest a lower probability of the occurrence of certain diseases (Sevinga et al. 2002; Johanson et al. 2003); however, it may also be due to different digestion mechanisms in ruminants and in perissodactyla.

In ruminants, the rumen and its structure, as well as continuous production of short-chain fatty acids (SCFA) play an important role in magnesium absorption. As shown by *in vitro* studies, particularly fatty acids with short chains are involved in increased transport of magnesium across the rumen epithelium (Gabel et al. 1993, Leonhard-Marek 1999). The coupling between butyrate and magnesium transport may by mediated by another mechanism, perhaps by co-transport of $Mg^{2+}$ and $HCO_3^-$ (Schweigel et al. 2000).

It may be assumed that the small difference in magnesium levels between serum and erythrocytes in the group of ruminants is due to easy availability of magnesium from the gastrointestinal tract (Abel et al. 1993; Care 1994; Leonhard-Marek 1999). In ruminants, magnesium transport is carried out predominantly in the rumen and is accomplished even at very low concentrations of this cation in the rumen. A decrease in magnesium resorption was

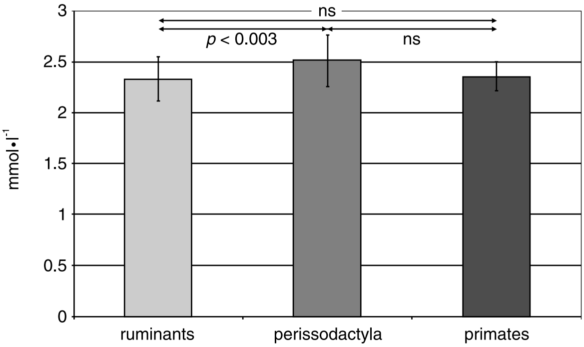


Fig. 3. Calcium concentrations in sera of the animal groups

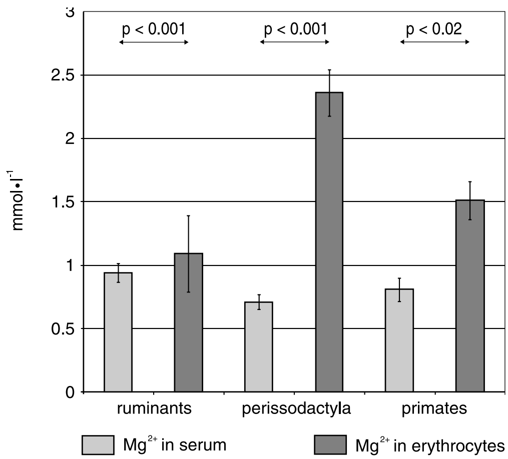


Fig 4. Magnesium concentrations in sera and erythrocytes of the animal groups

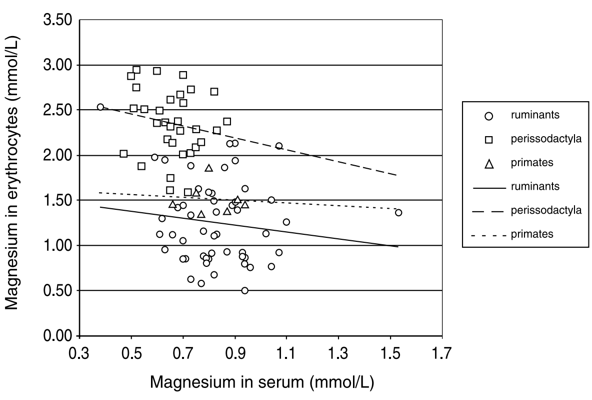


Fig. 5. The relationship of serum to erythrocyte concentrations in ruminants, perissodactyla and primates; the relationship is linear for each group

observed in the presence of higher potassium levels in the intestine and concomitant low intake of magnesium (Leonhard-Marek et al. 1996; Martens et al. 2003). When magnesium intake is increased, the negative effect of potassium on magnesium resorption is reduced. Other factors that are involed in magnesium resorption in ruminants include fermentation products such as $NH_4^+$ or SCFA (Martens 2000). Magnesium resorption is enhanced by SCFA presence in the rumen.

In perissodactyla, in contrast to ruminants, fermentation factors play a role – for instance, production of SCFAs in the colon. Similarly, magnesium absorption in perissodactyla is not markedly associated with the proximal part of the digestive system only, as it is in ruminants. This implies that magnesium resorption in perissodactyla can occur over a larger region of the digestive system that it does in ruminants and this may facilitate a higher supply of magnesium to the cells with high metabolic activity.

On the other hand, magnesium levels may provide indirect evidence of energy production in the form of ATP (magnesium is an integral part of its molecule) and it may correspond to different levels of physical activity in ruminants and perissodactyla. While ruminants, with the exception of the chamois or lama, move slowly and less dynamically, perissodactyla are characterized by quick and intensive movement.

Of all three groups, the highest calcium concentration was found in perissodactyla. No correlation was found between erythrocyte and serum concentrations of magnesium and serum concentration of calcium in either the ruminants or perissodactyla. Calcium is an important ion involved in muscle contraction and magnesium is necessary for energy production (ATP) and other physiological functions of the cell, including muscle relaxation.

At the same time, higher levels of magnesium in muscle tissue prevent the development of spasm and tetany of skeletal muscles; in the myocardium, they reduce the occurrence of arrhytmias and, in transmission of nerve impulses, magnesium stabilization of the cell membrane reduces adverse effects (Saris et al. 2000).

Therefore the key issue is by which method magnesium deficiency can be detected before the onset of clinical signs.

The reasons are simple – a marked deficiency of magnesium may be associated with serious pathological conditions (Garcia-Lopez 2001). High losses due to magnesium deficiency have been described in cattle that showed clinical signs reminiscent of clostridium infection, mycotoxicosis or severe enteritis (Donovan et al. 2004). Acute abdominal conditions have been reported in horses (Garcia-Lopez 2001; Sevinga et al. 2002). These conditions are easy to overlook because the symptomatology of the overall magnesium deficiency in the organisms is not, in the case of moderate deficiency, noticeably specific (Wijnberg et al. 2002; Stewart et al. 2004). For this reason, the study on selected animals in the Prague ZOO was carried out. Although it was not practicable to evaluate interspecies differences due to small numbers of animals available, it was nevertheless possible to assess every group as a whole.

The primate group had serum and erythrocyte concentrations similar to those found in humans. If we regarded the human values as a physiological range, the concentration found in the primates would be at the lower limit of this physiological standard. A logical question arises as to whether the primates have sufficient magnesium intake with their diet in comparison with not only ruminants but also perissodactyla. Serum concentrations may not provide an indication of the true magnesium content in the body as reliably as erythrocyte concentrations. Moreover, the analysis of erythrocyte magnesium is less demanding in terms of time than the loading magnesium test (Stewart et al. 2004).

On the basis of our results in human studies we suggest that, for the assessment of magnesium deficiency in the organism, measurement of serum levels is the least sensitive indicator; erythrocyte concentrations are more sensitive and the loading test is the most sensitive method for detecting magnesium deficiency (Wilhelm 2002; Wilhelm 2004).

One of the reasons for assessing the serum and erythrocyte concentrations of magnesium in animals kept in a ZOO was that such information is not currently available. The differences between serum and erythrocyte concentrations in the groups investigated were unexpected. We suggest that our results should be a basis for the assessment of magnesium concentrations under strictly defined conditions in other animal species, because this is the only way of finding out the physiological range of magnesium concentrations in serum and erythrocytes in animals kept in ZOO parks. The importance of magnesium for animal physiology should warrant such studies.

**Koncentrace hořčíku v séru a erytrocytu u přežvýkavců, lichokopytníků a primátů pražské ZOO**

Cílem práce bylo posoudit a zhodnotit koncentrace hořčíku v séru a erytrocytech u zdravých zvířat různých zoologických skupin pražské ZOO: přežvýkavci (R, n = 55), lichokopytníci (P, n = 32) a primáti (Pr, n = 7). Ze vzorků žilní krve byly stanoveny hodnoty hematokritu a sérové koncentrace hořčíku (sMg). Současně byly stanoveny hodnoty hořčíku v plné, hemolyzované krvi a byly dopočteny hodnoty erytrocytárních koncentrací hořčíku (eryMg).

U jednotlivých skupin zvířat byly nalezeny následující sérové koncentrace hořčíku v mmol·l$^{-1}$ (R: 0,83 ± 0,16; P: 0,65 ± 0,09; Pr: 0,81 ± 0,09), statistické rozdíly byly zjištěny mezi přežvýkavci a lichokopytníky ($p < 0{,}001$), lichokopytníky a primáty ($p < 0{,}001$).

Erytrocytární koncentrace hořčíku (mmol·l$^{-1}$) byla u jednotlivých skupin zvířat (R: 1,25 ± 0,46; P: 2,35 ± 0,37; Pr: 1,51 ± 0,15), statistické rozdíly byly nalezeny mezi přežvýkavci a lichokopytníky ($p < 0{,}001$) a lichokopytníky a primáty ($p < 0{,}001$). U všech sledovaných skupin zvířat se koncentrace hořčíku v séru a erytrocytech signifikantně lišily v rámci skupiny (R: p < 0,001; P: p < 0,001; Pr: p < 0,02). Mezi hodnotami sérového a erytrocytárního hořčíku nebyly nalezeny korelace.Pro hodnocení tkáňových koncentrací hořčíku pokládáme jeho koncentraci v erytrocytech za klinicky významnější, než koncentraci v krevním séru.

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