MAGNESIUM METABOLISM IN CHILDHOOD

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ISBN 90-9004412-4

Dit onderzoek werd gesubsidïeerd door de Nierstichting.

Publicatie van dit proefschrift werd mede mogelijk gemaakt door giften uit het Fonds Wetenschapsbeoefening van de afdeling Kindergeneeskunde, het Fonds Wetenschapsbeoefening van het Centraal Klinisch Chemisch Laboratorium en de Nierstichting.

MAGNESIUM METABOLISM IN CHILDHOOD

EEN WETENSCHAPPELIJKE PROEVE OP
HET GEBIED VAN DE MEDISCHE WETENSCHAPPEN,
IN HET BIJZONDER DE GENEESKUNDE

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR AAN DE
KATHOLIEKE UNIVERSITEIT TE NIJMEGEN,
VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN IN HET
OPENBAAR TE VERDEDIGEN OP

WOENSDAG 23 OKTOBER 1991

DES NAMIDDAGS TE 1.30 UUR PRECIES

DOOR

WILHELMUS BERNARDUS GEVEN

GEBOREN OP 31 JANUARI 1951
TE DINXPERLO

Druk: Krips Repro Meppel

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ABBREVIATIONS

ADH	Anti diuretic hormone
ATP	Adenosyl triphosphate
bo-Mg	Bone magnesium content
C_K	Clearance of potassium
C_{H2O}	Free water clearance
$C_{H2O} + C_{Cl}$	Sum of free water clearance and clearance of chloride
DDAVP	1-Deamino(8-D-arginine)vasopressin
dens	Density of 1 ml red blood cells
2,3-DPG	2,3-Diphospho glycerate
dr. wt	Dry weight of 1 ml red blood cells
er-K	Erythrocyte potassium content
er-Mg	Erythrocyte magnesium content
fg	Femtogram
fw	Cell water fraction
GFR	Glomerular filtration rate
Lys-$[MgT]_i$	Total intracellular magnesium content after lysing erythrocytes
$[Mg^{2+}]_i$	Ionized free intracellular magnesium concentration
$[MgT]_i$	Total intracellular magnesium concentration
$[Mg^{2+}]_o$	Ionized magnesium concentration in the medium at equilibrium
mo-Prot	Mononuclear protein content
mo-DNA	Mononuclear desoxyribonucleinic acid content
mo-K	Mononuclear potassium content
mo-Mg	Mononuclear magnesium content
mu-Mg	Muscular magnesium content
ng	Nanogram
pg	Picogram
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
6-keto-$PGF_{2\alpha}$	6-keto-Prostaglandin $F_{2\alpha}$
pl-Mg	Plasma magnesium concentration
^{31}P NMR	^{31}Phosphorous nuclear magnetic resonance
PRA	Plasma renin activity
RBC	Red blood cells
SA	Surface area
se-Mg	Serum magnesium concentration
TBX B_2	Tromboxane B_2
uf-Mg	Ultrafiltrable magnesium concentration in plasma
Uosmol	Urinary osmolality
ZPT	Zeropoint titration

Chapter 1

INTRODUCTION

Although various magnesium salts were used as cathartics already in the Renaissance in Italy, it lasted till the 1920s that magnesium first was shown to be essential for animals [1]. Clinical magnesium depletion was first described in 1934 in a small number of patients with various underlying diseases [2]. The clinical features may vary widely and depends on duration and gravity of the depletion.

The development of atomic absorption spectrophotometry in the 1950s and the numerous studies performed thereafter, accumulated to the conclusion that magnesium is not a trace element but the second most abundant intracellular cation. The total magnesium content of a human adult is about 1 mol elementary magnesium (24 grams), of which 50-60% is located in the bone and the rest distributed equally between muscle and other soft tissues [3].

DISTRIBUTION OF MAGNESIUM IN THE BODY

Serum

Only 1% of the total body magnesium is present in serum with a normal serum magnesium level of 0.70-1.1 mmol/l. Younger persons tent towards higher serum magnesium levels (Fig. 1). In serum about 34% of the magnesium content is bound to proteins, 11% complexed mainly to phosphates and citrates and 55% is present in the free ionized form [3].

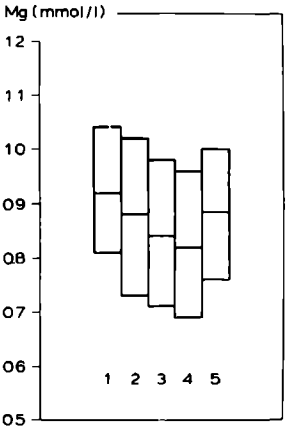

Figure 1. Serum magnesium concentration versus age; 1: < 2 years, 2: 2 - 10 years, 3: 10 - 20 years, 4: 20 - 40 years, 5: > 40 years.

Bone

Magnesium ions are not incorporated directly into the apatite mineral structure of bone, but are concentrated in the hydration shell around each hydroxyapatite crystal.

From this surface-bound ion complex magnesium can be released when magnesium intake is restricted [4]. The extent of this labile reserve varies with age and falls from about 30% of the total bone magnesium in young rats to 17% in adult animals [5]. In human bone 30% of the total magnesium is present in a surface limited pool, either dissolved in the hydration shell or absorbed on the surface of the hydroxyapatite crystals. This part of the bone magnesium is fairly rapidly exchangeable, whereas the remaining 70% forms an integral part of the bone crystal [6]. Trabecular bone contains more magnesium than cortical bone [6]. Alfrey et al. [7] claimed that there existed a highly significant correlation between levels of serum and bone magnesium both decreasing during magnesium depletion.

Muscle

In skeletal muscle fibres magnesium may bind to several proteins, such as troponin, calmodulin, parvalbumin, myosin and ATP-ases which are of great importance for an adequate muscle function [8]. Within muscle cells, like other nucleated cells, magnesium is not distributed homogeneously in the cell but has the highest concentrations in mitochondria and sarcoplasmatic reticulum. Most of total intracellular magnesium, concentration 3-6 mmol/l, is bound to proteins and only 0.6 mmol/l is present in the free ionized form [9]. In contrast to rats and dogs where no consistent significant decrease was noticed [10,11], a variable amount of the human skeletal-muscle magnesium can be lost with an average of 15%, equivalent to 0.55-0.99 mmol/kg [11]. The largest losses (40%) were noticed in children with protein-calorie malnutrition [12].

Mononuclear cells

In 1969 Baron and Ahmed [13] advocated leucocytes for the measurement of intracellular cations. Recently Elin [14] reviewed 10 years of human mononuclear blood cells magnesium measurements and concluded that mononuclear magnesium measurement is helpful in the assessment of the magnesium status of the patient. A significant correlation for magnesium in mononuclear cells and muscle was shown [15] and also for magnesium and potassium in mononuclear cells [16,17]. Normal values (mean ± 1 sd) for mononuclear magnesium are: 3.48 ± 1.28 fmol/cell (equivalent to 84 ± 31 fg/cell) or 53.3 ± 12.5 μmol/g protein (equivalent to 1.28 ± 0.30 μg/mg protein) [18].

Red blood cells

After Archer et al. [19] presented a method for the measurement of erythrocyte magnesium content, many reports were published. Normal values in adults are 1.69 - 2.99 mmol/l red blood cells [19]. Erythrocyte magnesium decreases with the age of the red blood cells and in magnesium deprivation erythrocytes have a shorter survival. The usefulness of red blood cell magnesium for clinical medicine remains unclear [20]. It is discussed later, highlighted by our own results.

Intracellular magnesium

As a necessary cofactor in more than 200 hundred enzymes, intracellular magnesium

influences a wide range of cellular functions such as glycolysis, respiration, protein-, DNA-, RNA-synthesis and transmembrane transport of other ions for example Na^+ and Ca^{2+} [21,22]. Modulation of calcium channel activity by magnesium in ventricular cells was found by Agus et al. [23].
Intracellular magnesium is compartmentalized and most of the magnesium, expressed as percentage of the total, was found in microsomes, followed by mitochondria and nucleus [24,25].

Table 1. Intracellular compartmentalisation of magnesium.

	% of total Mg [25]	% of total Mg [26]
nuclei	16.3	13.4
mitochondria	23.2	21.8
microsomes	45.2	48.0
supernatant(microsol)	14.1	12.8

In the different cell organelles magnesium is further compartmentalized; in mitochondria magnesium is localized for 4% at the outer membrane, 50% in the intermembranous space, 5% in the inner membrane and 41% is localized in the matrix [26]. In the cytosol 90% of magnesium is bound to ATP and other magnesium binding ligands, representing a intracellular magnesium buffer.

Intracellular free magnesium

Of the total intracellular magnesium less than 10% is present in the free ionized form, which is considered to be the metabolic active magnesium. Different techniques were used; metallochromic indicators, ^{31}P NMR, nullpoint titration and ion-selective electrodes [27]. A wide range of $[Mg^{2+}]_i$ is reported, depending on technique and investigator, but most probable estimates are in the range of 1 mmol per litre cell water. This is near the K_m of many enzymes [28]. As the $[Mg^{2+}]_i$ is hold constant by very efficient cellular mechanisms, it is not likely that magnesium has a trigger function but must be regarded more a static than a dynamic regulator.

MAGNESIUM TRANSPORT IN THE RED BLOOD CELL

Erythrocytes lack intracellular compartments and therefore can more easily be used for magnesium transport studies. Of the total erythrocyte magnesium content only about 10% is present in the free ionized form with a concentration of 0.25 - 0.40 mmol/l cell water [29,30]. With a plasma ionized magnesium concentration of about 0.5 mmol/l and a membrane potential of 9 mV inside (negative), the predicted intracellular magnesium concentration should be ≈1 mM at electrochemical equilibrium [31]. In order to maintain the considerably lower measured values, red

cells must be impermeable to magnesium or must actively transport magnesium out of the cell.

Magnesium influx

Experiments using ^{28}Mg showed very slow uptake of magnesium into human erythrocytes [32]. After 5 hours incubation with ^{28}Mg added to the plasma only approximately 5% of the relative activity was detected in the erythrocytes. Ginsburg et al. could not detect any uptake of ^{28}Mg by human red blood cells after 12 and 24 hours [33]. Later, Watson and co-workers found an uptake of ^{28}Mg into erythrocytes of 0.14 mmol/day, corresponding with approximately 60 μmol/l erythrocytes per day [34] while total magnesium content ranges from 1.69 - 2.99 mmol/l erythrocytes [19]. Due to the inwardly directed electrochemical gradient for magnesium, the influx into erythrocytes is considered to be passive.

Magnesium efflux

In a recent review Flatman [31] states that erythrocyte magnesium efflux is active and does not occur through the sodium pump, calcium pump, Na-K-Cl cotransporter, K-Cl cotransporter, or anion exchange system. Human red blood cells have a specialized sodium dependent magnesium transporter. However, Günther and Vormann [35] showed that only 10% of the maximal net magnesium efflux from human erythrocytes is dependent on extracellular sodium and that 90% depends on net chloride efflux for charge compensation.

MAGNESIUM HOMEOSTASIS

Intestinal resorption

The recommended daily intake of magnesium is 4-5 mg/kg of which 30-40% is absorbed in the intestinal tract (Fig. 2) [36]. After oral loading with ^{28}Mg the major part of absorption occurred during the first 6 hours, pointing to absorption in the small intestine [37]. In their recent review Hardwick et al. [38] concluded that magnesium is resorbed predominantly in the distal small intestine. Passive paracellular diffusion through the tight junctions, driven by the electrochemical gradient across the epithelium, accounts for the majority of magnesium absorbed [38]. Besides passive diffusion, solvent drag mechanisms occur, whereas active transport is of minor importance in the intestinal magnesium resorption.
The movement of magnesium across the basolateral membrane into the extracellular space is an active step.
Factors modifying the absorption of magnesium include dietary intake, dietary calcium (conflicting results), vitamin D and parathormone. Both vitamin D and parathormone augment intestinal magnesium absorption [39]. In suckling, weanling and adult rats a developmental character of intestinal magnesium transport was noticed by Meneely et al. [40]. Younger rats had higher intestinal absorption rates than older ones. In children absorption studies after oral ^{28}Mg gift and combined measurement of total

body retention and excretion of the isotope in urine and faeces revealed 24 - 32% intestinal absorption [41]. However absorption rates of 55 - 75% were also noticed [42]. Recently Schuette et al. [43] used ^{25}Mg, a stable isotope, to investigate the intestinal magnesium resorption in three full term infants. When 20 mg of ^{25}Mg was given over a 24 hour period a fractional resorption of 64.0% ± 3.9 was noticed [43]. This is nearly the doubled absorption rate reported by Lombeck et al. [41] but in the normal range of absorption found by Strømme et al. [42] using ^{28}Mg.

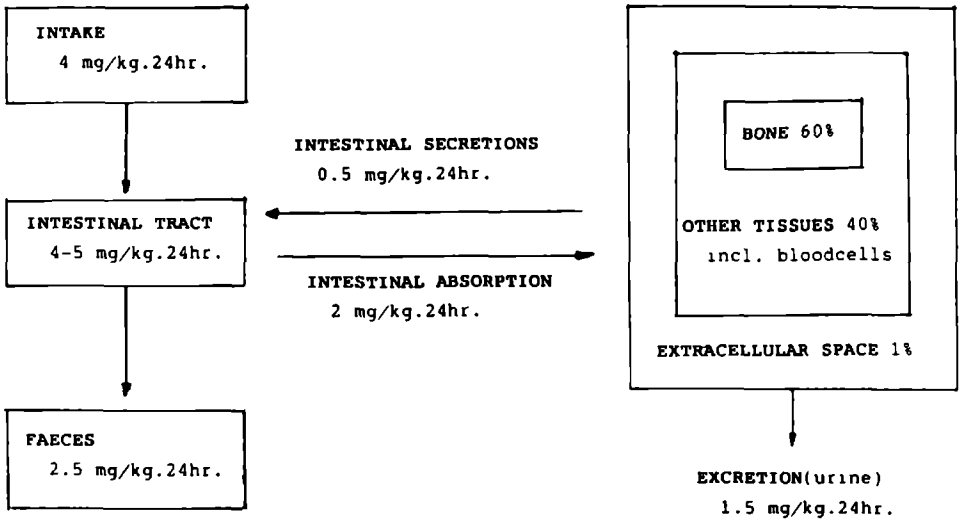

Figure 2. Body magnesium stores.

Interactions between calcium and magnesium metabolism

The relation between parathormone and magnesium levels are not straightforward. Acute lowering of serum magnesium seems to stimulate parathormone release, whereas chronic magnesium depletion is associated with lowered parathormone secretion [44]. Severe magnesium depletion can be attended with hypocalcemia. The combination of decreased parathormone release and bone resistance to parathormone might explain this association. In vivo and in vitro studies demonstrated inhibited PTH release during high magnesium concentrations [45,46].

Interactions between potassium and magnesium metabolism

Total body potassium in humans and animals is often reduced in magnesium deficiency [47]. During continuing magnesium depletion in humans, the accompanying intracellular potassium depletion cannot be restored with only extra supply of potassium, until magnesium is also replaced [48]. A decreasing intracellular magnesium content is associated with potassium efflux from the cell. In primary potassium depletion, intracellular magnesium can also be reduced. These interrelationships are not yet adequately explained.

RENAL HANDLING OF MAGNESIUM

Introduction

Ultrafiltrable magnesium as determined with the Amicon filter technique in plasma amounts to 0.60 ± 0.07 mmol/l (mean ± 1 sd). With a normal glomerular filtration rate of 120 ml/min, the filtered load can be calculated to be 104 mmol or 2500 mg/day. As the normal excretion in adults is 3 - 6 mmol/24 hours, this means that the urinary output represents about 5% of the filtered load of magnesium As magnesium absorption is essentially unidirectional 95% will be reabsorbed by the renal tubules.

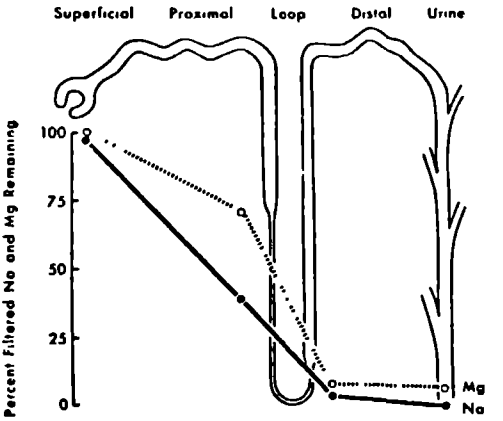

Figure 3 Summary of the fraction of filtered load of magnesium and sodium remaining along the proximal and distal tubules and final urine of normal dogs Fractions of filtered load are expressed as (TF/UF Na or Mg) / (TF/P inulin) x 100 From [49] with permission.

Renal magnesium reabsorption is clearly different from the reabsorption of sodium and calcium as is shown in figure 3. This figure illustrates the proximal and distal tubule-reabsorption and also the final urine of normal dogs [49]. The same scheme applies to all species investigated [50]. In contrast with a fractional reabsorption for sodium and calcium of 50-60% in the proximal tubules, some 20-30% of the filtered magnesium is reabsorbed in this part of the nephron. The major portion of

magnesium (≈ 65%) is reabsorbed within the loop of Henle mainly in the thick ascending limb. About 10% of the filtered magnesium reaches the distal nephron, where only a small fraction is reabsorbed.

Tubular reabsorption

As excellent reviews [49-53] about this aspects are available we will only briefly review the tubular reabsorption and stress the points needed to understand the content of this thesis.

Proximal convoluted tubule

Luminal magnesium concentration rises along the length of the proximal tubule to be 1.7 with respect to ultrafiltrated magnesium at a point where 50 to 55% of sodium and water had been reabsorbed. The proximal tubule epithelium possesses a low permeability for magnesium relative to sodium or calcium.
The segmental reabsorption of Mg, Ca and Na in normal animals is shown in table 2 The reabsorption rate is dependent on the luminal magnesium concentration as was shown using in vivo microperfusion technique.

Table 2. Segmental reabsorption of Mg, Ca and Na in normal animals.

					Fraction of filtered load remaining at collection site			
Collection site	TF/P_{inulin}	TF/P_{Na}	TF/UF_{Ca}	TF/UF_{Mg}	H_2O	Na^+	Ca^{2+}	Mg^{2+}
Glomerulus	1 0	1 0	1 0	1 0	100	100	100	100
Proximal convoluted tubule	2 5	1 0	1 2	2 0	40	40	48	80
Descending limb of Henle	4 5	2 5	2 5	4 0	22	55	55	90
Early distal tubule	5 0	0 4	0 4	0 6	20	8	8	12
Late distal tubule	10 0	0 25	0 25	0 8	10	2	2	8
Urine					1	0 5	0 5	5

Data represent mean approximations at the end of the respective accessible nephron segments
TF/P and TF/UF are tubular fluid/plasma or ultrafiltrable plasma concentration ratios [53]

Proximal straight tubule and thin descending limb of Henle's loop

In-vitro perfusion studies of isolated straight proximal segments of the rabbit showed the same characteristics for magnesium transport as in the proximal convoluted tubule. In the descending limb of Henle magnesium is added at the same time as water is abstracted.

Thick ascending limb of Henle's loop

This is the major segment of the nephron reabsorbing filtered magnesium. There is a dependence of magnesium transport rate with the load of Mg delivered to the loop of Henle, whether delivery is altered by increasing luminal Mg concentration or increasing flow rate. The increasing flow rate may cause Mg absorption to increase because the Mg concentration in tubular fluid increases along the length of the thick ascending limb. Elevation of luminal Mg 10-fold above normal has no effect on net sodium or calcium transport within the thick ascending limb. In contrast elevation of the plasma magnesium concentration at the basolateral membrane markedly depresses magnesium reabsorption. Net sodium absorption is not reduced by peritubular Mg but net calcium transport was significantly reduced (Fig. 4).
Hypercalcemia inhibits both magnesium and calcium reabsorption in the loop of Henle by an unknown mechanism.
The thick ascending limb comprises cortical (CTAL) and medullary (MTAL) portions. Both types of cells possess an electroneutral Na^+-$2Cl^-$-K^+ cotransporter at the apical side and an Na^+-K^+-ATPase at the basolateral membrane (Fig. 5).
The K^+ entering the cell is recycled through the apical K^+-channel. Cl^- is extruded from the cell via chloride conductance. The presence of a large potassium conduction across the luminal membrane and the Cl^- exit across the basolateral membrane provides the basis for the lumen-positive transepithelial potential.
Although the CTAL and MTAL have the same morphological organization, the cells of the cortical portion are narrower and have a simpler ultrastructural organization. There is also a clear functional heterogeneity. In the rat the adenylcyclase system of the CTAL and MTAL are both sensitive to ADH, glucagon and calcitonin. Only CTAL is sensitive to PTH.
In the mouse CTAL was sensitive to all tested hormones, ADH, glucagon, calcitonin and PTH whereas in MTAL the cyclase system was only stimulated by ADH and glucagon. Most important are the results of the effect of ADH on transepithelial Cl^-, K^+, Ca^{2+} and Mg^{2+} net transport in TAL performed in vitro.
ADH stimulated Ca and Mg reabsorption in the cortical part and Na^+ and Cl^- reabsorption in the medullary part of this nephron segment. In the normal situation the Ca and Mg resorption was almost limited to CTAL.
The problems of active and or passive transport in TAL is not completely solved. Mg^{2+} may pass through the paracellular pathway driven in a passive fashion by the transepithelial potential difference and active sodium transport. Alternatively Mg absorption may be active as well as transcellular in nature.
Glucagon and ADH increases Mg^{2+} transport with no alteration in Potential Difference, transepithelial resistance or NaCl absorption in CTAL of the mouse suggesting an active component for magnesium.
As is evident from Fig. 5 paracellular and transcellular magnesium absorption is related tot active Na^+ transport in TAL. An altered transepithelial voltage will influence an active and passive transport of magnesium. The transport of NaCl is normally greater in MTAL than in CTAL, but the CTAL maintains greater transepithelial concentration differences.

Distal convoluted tubule and collecting duct

Normally a small portion of magnesium is reabsorbed in the distal convoluted tubule. The reabsorption is close to its capacity whereas the calcium and sodium transport is normally unsaturated.

The collecting duct plays a very limited role in renal magnesiumtransport.

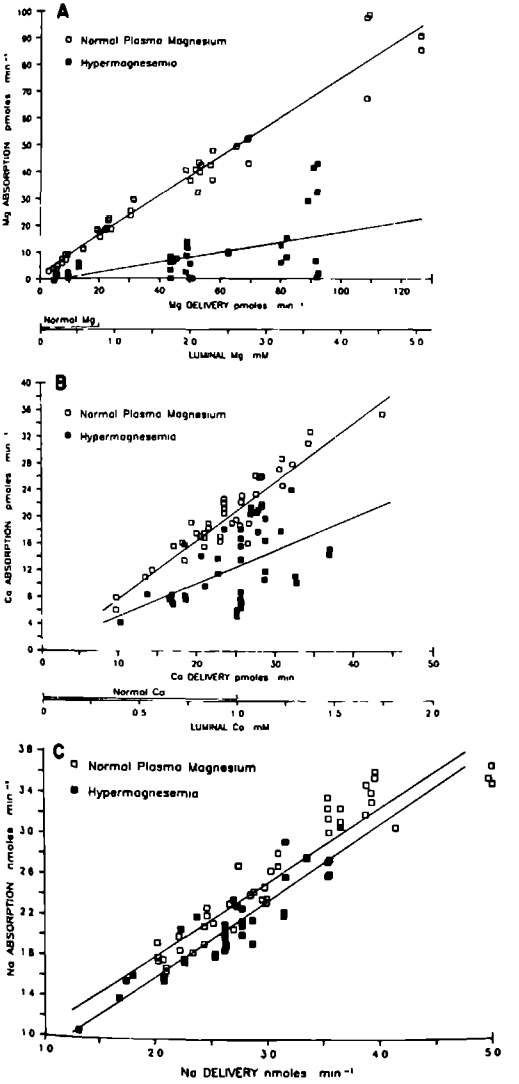

Figure 4. Effect of luminal magnesium versus peritubular magnesium concentration on net Mg, Ca and Na reabsorption in the loop of Henle. From [53] with permission.

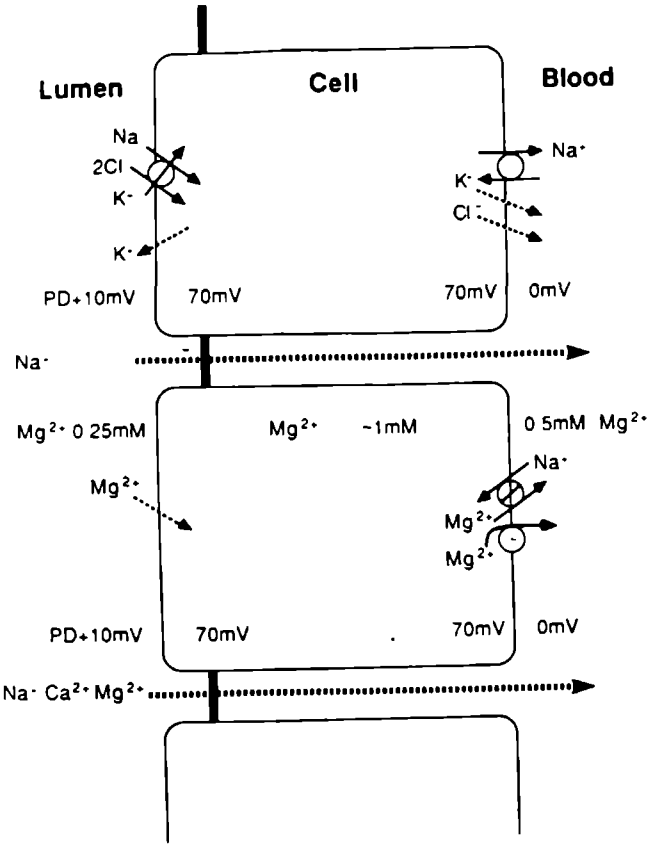

Figure 5. Schematic model of magnesium reabsorption in the thick ascending limb of Henle's loop Conductive pathways are denoted by dashed arrows and carrier-mediated transport by solid arrows Active transport processes are indicated by ~ symbol. From [53] with permission

DISTURBED MAGNESIUM HOMEOSTASIS

Many causes and factors of disturbed magnesium homeostasis leading to magnesium deficiency or hypomagnesemia are known.

Five main-groups can be formed (table 3) [54]; reduced intake, impaired intestinal resorption, excessive urinary losses, excessive losses of body fluids and intracellular shifts. In acute pancreatitis the hypomagnesemia might be caused by the precipitation of insoluble magnesium soaps in the omentum [55].

Although low serum magnesium concentrations usually imply intracellular magnesium depletion, intracellular magnesium depletion is reported despite normal serum magnesium levels. Conversely, low serum magnesium concentrations have been reported in patients with normal cellular magnesium content and no clinical signs of deficiency [56]. For many years there was uncertainty about the existence of a

Table 3. Mechanisms and causes of hypomagnesemia

I	Reduced intake
a	prolonged intravenous therapy without sufficient magnesium
b	starvation
c	protein calorie malnutrition
d	alcoholism
II	Impaired intestinal resorption
a	chronic diarrhoea
b	malabsorption syndromes
c.	primary hypomagnesemia
III	Excessive urinary losses
a	polyuria
b	hypercalcemia
c	drug-induced by furosemide, thiazides, gentamycin, amphoteri-cin B, cisplatin, cyclosporin, ifosfamide
d	Renal diseases -renal tubular acidosis -chronic glomerulo-pyelonephritis -specific tubular disorders
d	diabetes
e	hyperaldosteronism
f	hyperthyroidism
g	inappropriate antidiuretic hormone
h	alcoholism
i	potassium depletion
IV	Excessive losses of body fluids
a	prolonged nasogastric suction
b	intestinal fistula
c	excessive lactation
d	exchange transfusion with blood anti-coagulated with citrate
V	Intracellular shifts
a	postparathyroidectomy
b	refeeding after starvation
c	insulin treatment
d	correction of acidosis
e	acute pancreatitis

From D Juan [54]

pure magnesium deficiency state in man because hypomagnesemia is often accompanied by other electrolyte disturbances. The experimental magnesium deficiency study in man performed by Shils [47] showed that urinary magnesium excretion drops very quick and is below 1 mmol/day after 3 days of depletion. Serum magnesium lowers gradually and erythrocyte magnesium content follows the trend in serum with some delay. One or more signs of magnesium deficiency developed in 24 to 102 days in six of seven depleted persons.

Detection of magnesium deficiency

The incidence of magnesium deficiency is still subject of debate. In severe depletion a lowered serum magnesium level combined with a nearly absence of magnesium in the urine is found, but several authors report a normomagnesemic magnesium deficiency. In this situation urinary magnesium excretion is in the lower range
of normal, but not as low as in severely magnesium depleted patients. Increased magnesium retention was noticed during normal serum magnesium levels in alcoholics,

a situation with a known relation to magnesium depletion compared to controls [56]. Erythrocyte and mononuclear magnesium content are reported to be helpful in diagnosing magnesium deficiency but both have wide normal ranges and are not a standard tool at this moment.
Different types of magnesium loading tests are developed; urinary magnesium excretion before and after an intravenous or intramuscular load with magnesium in different dosages is measured and the magnesium retention calculated. Although

Table 4. Symptoms of magnesium depletion.

apathy	hallucinations
anorexia, nausea and vomiting	tremulousness
muscular weakness	hyperreflexia
spasmophillia	positive Chvostek sign
myophatic potentials on the electromyogram	positive Trousseau sign
low voltage T-wave and PQRS-complex, short PR-interval on the electrocardiogram	gross muscular tremor
	choriform movements
	ataxia
confusion	tetany
depression	vertigo
agitation	delirium
epileptiform convulsions	

urine collection is the cornerstone of these tests, the pre-load urine period, mostly eight hours, is rather short for adequate judgement of basic magnesium excretion. In prematures and newborns the low renal magnesium clearance due to the low glomerular filtration rate may lead to falsely high magnesium retention because of the relatively short period of post-load urine collection (32 hours) in the magnesium load test, designed by Byrne and Cadell [57].

ASPECTS OF DISTURBED MAGNESIUM HOMEOSTASIS IN CHILDHOOD

Decreased intake

Although causes and mechanisms of abnormalities in magnesium homeostasis are generally the same in adults and children, some differences especially in neonates and infants are noticed.
In neonates the magnesium content ranges from 7-10 mmol/kg body weight [58], serum levels are significantly lower on the first day [59] and increase considerably during the first three days after birth [60]. This rise of the serum magnesium level is accompanied by low urinary magnesium excretion of 0.34, 0.13 and 0.13 mg/kg body weight on the first, second and third day respectively [61].
Under standardised conditions with a magnesium intake of 10.5 ± 3.7 mg/kg.day in normal infants and children with ages from one month to 14 years, an urinary

excretion of 2.8 ± 1.1 mg/kg.day with a range from 0.9 - 5.2 was reported by Paunier et al. [62]. In a larger study, where home made food was ingested, urinary magnesium excretion was in the same range (2.03 ± 0.77 mg/kg.day) and a circadian rhythm noticed [63]. Significant higher magnesium (and calcium) excretion was found in overnight urine.
Common causes of neonatal hypomagnesemia are intrauterine growth retardation, where hypomagnesemia is considered to be a part of the syndrome with decreased placental transfer of magnesium or altered fetal magnesium metabolism [64], exchange transfusion with blood anti-coagulated with citrate, malabsorption syndromes such as biliary atresia, mostly after intestinal surgery, and maternal hypomagnesemia [61]. Decreased maternal magnesium intake, overuse of stoolsofteners and diabetes are also frequently noted causes [61].
Very rare is primary hypomagnesemia with secondary hypocalcemia characterized by recurrent tetany and convulsions [65]. Restoration of a normal serum magnesium level results in immediate cessation of the convulsions and a gradually normalisation of the serum calcium level. Primary impairment of the intestinal absorption of magnesium is the underlying defect. First symptoms are noticed from 9 days to four month after birth. Male to female ratio is 6 to 1 and the most likely mode of inheritance is autosomal recessive [66]. Livelong supplementation with high dose magnesium is necessary and prognosis appears good when the illness is diagnosed and treated promptly.

Increased magnesium loss

Increased magnesium losses leading to hypomagnesemia are reported in diuretic therapy and renal disorders. In table 3 the renal diseases resulting in hypomagnesemia are listed. The specific syndromes with renal magnesium loss will be discussed in more detail.
The diagnosis renal magnesium loss is established by the presence of hypomagnesemia with an inappropriately high 24 hour urinary magnesium excretion. Other causes of high urinary magnesium excretion must be excluded for example diabetes mellitus, the use of osmotic agents and diuretics [67]. Many of these renal magnesium loosing syndromes are hereditary disorders, but also isolated patients are reported [68]. Hypomagnesemia is often not the only abnormality noticed in these patients; combinations with hypokalemia, hypocalcemia, impaired concentration capacity of the kidney and/or nefrocalcinosis are frequently reported.
Rodríguez-Soriano [69] distinguishes three different types of hereditary renal magnesium loss: Isolated familial hypomagnesemia, familial hypokalemia-hypomagnesemia and familial hypomagnesemia-hypercalciuria.
Isolated familial hypomagnesemia was first reported in 1966 by Freeman and Pearson [70] in a woman and one of her five children. The absence of hypokalemia or nephrocalcinosis separates this entity from the two other entities. Two new families are described in this thesis.
Patients with Familial hypokalemia-hypomagnesemia, also known as Gitelman's syndrome [71] are often asymptomatic but transient episodes of muscular weakness and tetany are reported. There is no growth retardation, or polyuria. Urinary calcium

excretion is low (0.3 ± 0.1 mg/kg.day) and discrimination from Bartter's syndrome is sometimes difficult. This point will be treated in chapter 9.
Of the familial hypomagnesemia-hypercalciuria syndrome until now 18 patients are reported [69]; the absence of growth retardation, hypokalemia, and a tendency to metabolic acidosis distinguishes them from Bartter's syndrome patients. Hypomagnesemia is always accompanied by hyperuricemia and nephrocalcinosis. Ocular abnormalities such as myopia and horizontal nystagmus are often present. Defects in concentrating capacity and distal urinary acidification are probably secondary to nephrocalcinosis.

Hypermagnesemia

Cardiac conduction is affected at serum concentrations of 2.5 to 5.0 mmol/l, resulting in increases in the PR interval and QRS duration. Hypotension may occur. Deep tendon reflexes are lost when serum magnesium concentration approaches 5 mmol/l and respiratory arrest occurs near 7.5 mmol/l. Extremely high serum concentrations (>12.5 mmol/l) cause cardiac arrest [72]. In adults, hypermagnesemia is mostly seen in renal insufficiency when glomerular filtration rate falls below 30 ml/min. Suppletion with magnesium to prevent hyperphosfatemia can have an additional effect in renal insufficiency.
In neonates hypermagnesemia mostly is induced by maternal hypermagnesemia due magnesium sulphate infusions in pre-eclampsia [60]. The low glomerular filtration rate present in newborns causes a slow return to normal serum magnesium values.

THE AIM OF THIS THESIS

The origin of this investigation was based, as often in clinical medicine, on a clinical observation.
A family with isolated magnesium wasting was studied. It appeared to be an autosomal dominant form of renal magnesium loss. Although this disorder was already mentioned in the literature, it was not established as an entity. Another family with isolated renal magnesium loss and autosomal recessive form of inheritance was also observed. It was striking that although the level of serum magnesium was distinctly decreased, they had almost no clinical symptoms.
What was the relation between a lowered serum magnesium and the magnesium body stores?
An experimental study was planned in dogs. They received a magnesium deficient diet during several weeks. Neither the leucocyte content neither the bone content of magnesium but the content of erythrocyte magnesium appeared to be a good reflection of body magnesium stations (Chapter 6). The rate of decrease of erythrocyte magnesium compared with serum magnesium was studied in chapter 6 and 7. As the "metabolic" active form of intracellular magnesium is ionized magnesium, ionized magnesium was measured by the NMR technique, in erythrocytes of the experimental dogs and in patients with disturbances in tubular magnesium reabsorption (chapter 7 and 8). To validate the NMR technique, the results of the

NMR technique in erythrocytes was compared with the results of the zeropoint titration method (chapter 8).
The developed techniques were ultimately applied in patients with tubular disorders with possible magnesium depletion and described in chapter 8 and chapter 9.

REFERENCES

1 Leroy, J Nécessité du magnesium pour la croissance de al souris Compte Rendus des Seances de la Société de Biologie 1926, 94 431 433
2 Hirschfelder, A.D, Haury, V G Clinical manifestations of high and low plasma magnesium dangers of epson salt purgation in nephritis J Am Med Assoc 1934, 102 1138-1141
3 Wacker, W E C Magnesium and man Harvard University Press Cambridge, Massachusetts, London 1980
4 Brautbar, N, Gruber, H E Magnesium and bone disease Nephron 1986, 44 1-7
5 Heaton, F W Magnesium relations with parathyroid hormone, calcitonin and bone Magnesium-Bull 1981, 1a 67-72
6 Alfrey, A.C, Miller, N L. Bone magnesium pools in uremia J Clin Invest 1973, 52 3019 3027
7 Alfrey, A.C., Miller, N L, Butkus, D Evaluation of body magnesium stores J Lab Clin Med 1974, 84 153-162
8. López, J R, Alamo, L., Caputo, C, Vergara, J, DiPolo, R Direct measurement of intracellular free magnesium in frog skeletal muscle using magnesium-selective microelectrodes Biochim Biophys Acta 1984, 804 1-7
9 Gupta, R K., Moore, R D ^{31}P NMR Studies of intracellular free Mg^{2+} in intact frog skeletal muscle J Biol Chem 1980, 255 3987-3993
10 Cronin, R E, Ferguson, E R, Shannon, W A., Knochel, J P Skeletal muscle injury after magnesium depletion in the dog Am J Physiol 1982, 243(Renal Fluid Electrolyte Physiol 12) F113-F120
11 Wallach, S Magnesium exchangeability and bioavailability in magnesium deficiency In Magnesium in cellular processes and medicine 4th int symp on magnesium, Blacksburg, Va 1985 Eds Altura, B, Durlach, J, Seelig, M Karger, Basel 1987, pp 27-49
12 Cadell, J L, Goddard, D R Studies in protein calorie malnutrition New Engl J Med 1967, 276 533-535
13 Baron, D N, Ahmed, S A. Intracellular concentrations of water and the principal electrolytes determined by analysis of isolated human leucocytes Clin Sci 1969, 37 205-219
14 Elin, R J Status of the determination of magnesium in mononuclear cells in humans Magnesium 1988, 7 300-305
15 Sjögren, A., Florén, C-H, Nilsson, A. Magnesium and potassium status in healthy subjects as assessed by analysis of magnesium and potassium in skeletal muscle biopsies and magnesium in mononuclear cells Magnesium 1987, 6 91-99
16 Giardin, E, Paunier, L Relationship between magnesium, potassium and sodium concentrations in lymphocytes and erythrocytes from normal subjects Magnesium 1985, 4 188-192
17 Abraham, S A., Rosenman, U E D, Meshulam, Z., Brisk, R Lymphocyte and erythrocyte concentration of potassium, magnesium and calcium in normal controls Magnesium 1985, 4 102-105
18 Hosseini, J M, Johnson, E, Elin, R Comparison of two separation techniques for the determination of blood mononuclear cell magnesium content J Am Coll Nutr 1983, 4 361-368
19 Archer, W H, Emerson, R L., Reusch, C S Intra and extracellular fluid magnesium by atomic absorption spectrophotometry Clin Biochem 1972, 5 159 161
20 Elin, R Overview of problems in the assessment of magnesium status In Magnesium in cellular processes and medicine 4th int symp on magnesium, Blacksburg, Va 1985 Eds Altura, B, Durlach, J, Seelig, M Karger, Basel 1987, pp 67-76
21 Gunther, T Functional compartmentalisation of intracellular magnesium Magnesium 1986, 5 53-59

22. Gunther, T., Vormann, J., Förster, R. Functional compartmentalisation of intracellular magnesium Magnesium-Bull 1984, 2. 77-81.
23 Agus, Z.A., Kelepouris, E., Dukes, I , Morad, M Cytosolic magnesium modulates calcium channel activity in mammalian ventricular cells Am. J. Physiol 1989, 256· C452-C455
24. George, G.A., Heaton, F W. Changes in cellular composition during magnesium deficiency. Biochem J. 1975; 152: 609-615.
25. Griswold, R.L., Pace, N. The intracellular distribution of metal ions in rat liver. Expl Cell. Res 1956; 11: 362-367.
26. Bogucka, K., Wojtczak, L. Binding of magnesium by proteins of the mitochondrial intermembrane compartment. Biochem. Biophys. Res Commun. 1976. 71: 161-167.
27. Alvarez-Leefmans, F.J., Giraldezs, F., Gamino, S.M Intracellular free magnesium in excitable cells. its measurement and its biologic significance Can. J Physiol. Pharmacol 1987, 65 915-925
28. Gunther, T. Biochemistry and pathobiochemistry of magnesium Magnesium Bull 1981, 1a: 91-101.
29. Flatman, P.W., Lew, V.L. Magnesium buffering in intact human red blood cells measured using the ionophore A23187. J. Physiol. 1980, 305: 13-30.
30. Gupta, R.K., Benovic, J.L., Rose, Z.B The determination of the free magnesium level in the human red blood cell by ^{31}P NMR. J. Biol. Chem. 1978, 253 6172-6176
31. Flatman, P.W. The control of red cell magnesium Magnesium-Res 1988, 1· 5-11
32 Rogers, T.A. The exchange of radioactive magnesium in erythrocytes of several species J Cell Comp. Physiol 1961; 57 119-121.
33 Ginsburg, S , Smith, J.G., Ginsburg, F M , Reardon, J Z., Aikawa, J K. Magnesium metabolism of human and rabbit erythrocytes. Blood. 1962, 20. 722-729
34. Watson, W.S., Hildith, T E , Horton, P.W , Davies, D.L , Lindsay, R Magnesium metabolism in blood and the whole body in man using ^{28}Magnesium Metabolism 1979, 28 90-95
35. Gunther, T., Vormann, J. Na^+-independent Mg^{2+} efflux from Mg^{2+}-loaded human erythrocytes Febbs Letters 1989, 247: 181-184
36. Thorén, L. Magnesium metabolism Progr Surg 1971, 9 131-156
37. Danielson, B.G., Johansson, G , Jung, B , Ljunghall, S., Lundqvist, H , Malmborg, P. Gastrointestinal magnesium absorption Mineral Electrolyte Metab 1979, 2 116-123
38. Hardwick, L.L., Jones, M.R., Brautbar, N., Lee, D B N Site and mechanism of intestinal magnesium absorption Mineral Electrolyte Metab 1990, 16. 174-180.
39 Levine, B.S., Brautbar, N , Walling, M W., Lee, D B N , Coburn, J W. Effects of vitamin D and diet magnesium on magnesium metabolism J Physiol. (Endocrinol Metab 2) 1980, 239 E515-E523
40. Meneely, R., Leeper, L., Ghishan, F.K Intestinal maturation. in vivo magnesium transport Pediatr Res. 1982, 16· 295-298.
41 Lombeck, I and Bremer, H J. Primary and secondary disturbances in trace element metabolism connected with genetic metabolic disorders. Nutr. Metab. 1977, 21 49-64.
42. Strømme, J H , Nesbakken, R., Normann, T , Skjorken, F , Skyberg, D , Johannessen, B Familial hypomagnesaemia. Acta Paediatr. Scand 1969, 58: 433-444
43. Schuette, S.A., Ziegler, E.E , Nelson, S.E., Janghorbani, M. Feasibility of using the stable isotope ^{25}Mg to study Mg metabolism in infants. Pediatr. Res 1990, 27 36-40
44. Anast, C.S., Winnacker, J.L., Forte, L.R., Burns, T.W. Impaired release of parathyroid hormone in magnesium deficiency. J. Clin Endocrinol. Metab. 1976, 42. 707-717.
45. Buckle, R.M., Care, A.D., Cooper, C W., Gitelman, H J The influence of plasma magnesium on parathyroid hormone excretion. J. Endocrinol. 1968, 42: 529-534
46. Sherwood, L.M., Herrman, I., Basset, C.A. Parathyroid hormone secretion in vitro. regulation by calcium and magnesium ions. Nature. 1970, 225: 1056-1058
47. Shils, M.E. Experimental production of magnesium deficiency in man. Ann N Y. Acad. Sci 1969, 162: 847-855
48. Whang, R., Flink, E.B , Dyckner, T., Wester, P.O., Aikawa, J.K., Ryan, M.P. Magnesium depletion as a cause of refractory potassium repletion. Arch. Intern. Med. 1985, 145 1686-1689.
49. Quamme, G.A., Wong, N.L.M., Dirks, J.H., Rainel, N., De Rouffignac, C , Morel, F Magnesium handling in the dog kidney: a micropuncture study. Pflugers Arch. 1978, 377: 95-99.
50. Quamme, G A., Dirks, J.H. Magnesium transport in the nephron. Am J. Physiol (Renal Fluid Electrolyte Physiol. 8) 1980, 239. F393-F401.

51. Alfrey, A.C. Disorders of magnesium metabolism. In: The kidney: physiology and pathophysiology, ed. Seldin, D.W. and Giebisch, G. Raven Press, New York. 1985:1281-1295.
52. De Rouffignac, C., Elalouf, J-M., Roinel, N. Physiological control of the urine concentrating mechanism by peptide hormones. Kidney Int. 1987; 31: 611-620.
53. Quamme, G.A. Control of magnesium transport in the thick ascending limb. Am. J. Physiol.(Renal Fluid Electrolyte Physiol. 25) 1989; 256: F197-F210.
54. Juan, D. Clinical Review: the clinical importance of hypomagnesemia. Surgery. 1982; 91: 510-517.
55. Edmondson, H.A., Berne, C.J., Homann Jr., R.E., Wertman, M. Calcium, potassium, magnesium and amylase disturbances in acute pancreatitis. Am. J. Med. 1952; 12: 34-42.
56. Ryzen, E., Elbaum, N., Singer, F.R., Rude, R.K. Parenteral magnesium tolerance testing in the evaluation of magnesium deficiency. Magnesium. 1985; 4: 137-147.
57. Byrne, P.A., Cadell, J.L. The magnesium load test: II. Correlation of clinical and laboratory data in neonates. Clinical Pediatrics. 1975; 14: 460-465.
58. Forbes, G.B. Methods of determining composition of the human body. Pediatrics. 1962; 29: 477-494.
59. Nelson, N., Finnström, O., Larsson, L. Neonatal hyperexcitability in relation to plasma ionized calcium, magnesium, phosphate and glucose. Acta Paediatr. Scand. 1987; 76: 579-584.
60. Chan, G.M., Nordmeyer, F.R., Richter, B.E., Ash, K.O. Comparison of serum total calcium, dialysable calcium and dialysable magnesium in well and sick neonates. Clin. Physiol. Biochem. 1984; 2: 154-158.
61. Tsang, R.C. Neonatal magnesium disturbances. Amer. J. Dis. Child. 1972; 124: 282-293.
62. Paunier, L., Borgeaud, M., Wyss, M. Urinary excretion of magnesium and calcium in normal children. Helv. Paed. Acta. 1970; 25: 577-584.
63. DeSanto, N.G., DiIorio, B., Capasso, G., Giordano, D.R., Aulisio, M., Paduano, C., Stamler, J. Circadian rhythm with acrophase at night for urinary excretion of calcium and magnesium in childhood: Population-based data of the Cimitile study in southern Italy. Mineral Electrolyte Metab. 1988; 14: 235-239.
64. Tsang,R.C., Oh, W. Serum magnesium levels in low birth weight infants. Amer. J. Dis. Child. 1970; 120: 44-48.
65. Yamamoto, T, Kabata, H., Yagi, R., Takahima, M., Itokawa, Y. Primary hypomagnesemia with secondary hypocalcemia. Report of a case and review of the world literature. Magnesium. 1985; 4: 153-164.
66. Abdulrazzaq, Y.M., Smigura, F.C., Wettrell, G. Primary infantile hypomagnesaemia; report of two cases and review of the literature. Eur. J. Pediatr. 1989; 148: 459-461.
67. Evans, R.A., Carter, J.N., George, C.R.P., Walls, R.S., Newland, R.C., McDonnell, G.D., Lawrence, J.R. The congenital magnesium-losing kidney. Quat. J. Med. 1981; 197: 39-52.
68. Runneberg, L., Collan, Y., Jokinen, E., Lähdevirta, J., Aro, A. Hypomagnesemia due to renal disease of unknown etiology. Am. J. Med. 1975; 59: 873-881.
69. Rodríguez-Soriano, J., Vallo, A., García-Fuetes, M. Hypomagnesaemia of hereditary renal origin. Pediatr. Nephrol. 1987; 1: 465-472.
70. Freeman, R.M., Pearson, E. Hypomagnesemia of unknown etiology. Am. J. Med. 1966; 41: 645-656.
71. Gitelman, H.J., Graham, J.B., Welt, L.G. A new familial disorder characterized by hypokalemia and hypomagnesemia. Trans. Assoc. Am. Physicians. 1966; 79: 221-233.
72. Vernon, W.B., Wacker, W.E.C. Magnesium metabolism. In: Recent advances in clinical biochemistry, ed. Alberti, K.G.M.M. Churchill Livingstone Edinburgh. 1978: 39-71.

RENAL MAGNESIUM WASTING IN TWO FAMILIES WITH AUTOSOMAL DOMINANT INHERITANCE

Published in: Kidney International 1987; 31: 1140-1144.

RENAL MAGNESIUM WASTING IN TWO FAMILIES WITH AUTOSOMAL DOMINANT INHERITANCE

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SUMMARY

Renal magnesium wasting in two families with autosomal dominant inheritance. Hypomagnesemia due to isolated renal magnesium loss was demonstrated in two unrelated families with autosomal dominant mode of inheritance. Magnesium infusions performed in two patients showed not only a reduced renal magnesium threshold but also a lowered renal tubular maximum for magnesium. All members of both families who presented with hypomagnesemia had also a lowered excretion of calcium in the urine, presumably as a consequence of increased reabsorption in Henle's loop.

INTRODUCTION

Magnesium depletion due to congenital impairment in tubular reabsorption of magnesium is uncommon. Generally it is part of a more extensive tubular disturbance [1]. Isolated magnesium loss by the kidney appears to be extremely rare. The aim of this study is to report renal magnesium wasting in two unrelated families with autosomal dominant mode of inheritance. All family members who presented with hypomagnesemia, had also a lowered urinary excretion of calcium presumably as a consequence of hypomagnesemia.

METHODS

Case histories

M.C., the second child of an apparently normal family, was admitted at the age of 13 years because of generalized convulsions. After symptomatic treatment she complained of hallucinations and anxiety during one week. She had no previous

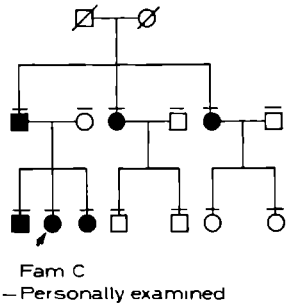

Figure 1. Pedigree of family C. Roman numerals for the different generations. Every generation member is designated by an Arabic numeral from the left to the right. Autosomal dominant mode of inheritance is presented. Arrow (↗) indicates patient M.C.; (–) indicates patient personally examined.

medical problems with the exception of some circumoral numbness. Extensive neurologic investigations revealed no abnormality (CAT-scan, cerebral angiography). A low serum magnesium (0.40 mmol/liter) was measured (normal 0.75 to 1.25 mmol/liter). Laboratory data revealed a normal serum sodium 138 to 141 mEq/liter, a normal serum potassium 3.8 to 4.2 mEq/liter, a normal serum chloride 101 to 105 mEq/liter, normal serum bicarbonate 26.2 mEq/liter, normal blood pH 7.36, normal serum calcium 2.45 mmol/liter, and normal ionized calcium 1.23 mmol/liter. Plasma renin activity and plasma aldosterone were in the normal range [2]. Parathormone level was 7.6 pmol/liter (normal <10). Calcitonin concentration was 62 pg/ml (normal 47 ± 24 pg/ml). Serum magnesium measurements performed in other members of the family indicated an autosomal dominant mode of inheritance (Fig. 1). The renal function and magnesium metabolism was more extensively studied in this girl.

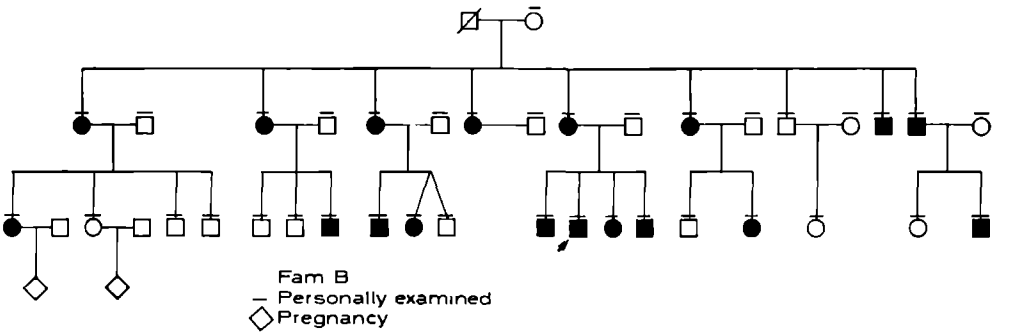

Figure 2. Pedigree of family B Roman numerals for the different generations while every generation member is designated by an Arabic numeral from the left to the right Autosomal dominant mode of inheritence is shown. Arrow (↗) indicates patient J B ,(–) indicates patients who were personally examined, (◇) indicates pregnancy.

J.B., the second child out of four children had an uneventful neonatal period after normal pregnancy. At the age of seven months he presented with generalized convulsions. Since that time he was treated by several anti-epileptic drugs. Severe psychomotoric retardation became evident. At the age of 15 years serum magnesium was measured for the first time and amounted to 0.39 mmol/liter. Routine laboratory data showed a normal serum sodium 137 to 145 mEq/liter, a normal serum potassium 3.9 to 4.2 mmol/liter, a normal serum chloride 104 mmol/liter, a normal serum bicarbonate 24.2 mmol/liter, normal blood pH 7.40, normal serum calcium 2.45 mmol/liter, and normal ionized calcium 1.26 mmol/liter. Plasma renin activity, plasma aldosterone, plasma parathormone level (6.3 pmol/liter) and plasma calcitonin concentration (47 pg/ml) were in the normal range. Studies of serum magnesium levels in other members of the family demonstrated an autosomal dominant mode of inheritance (Fig. 2). Also in this boy the renal function and magnesium metabolism was more completely studied. His severe mental retardation prohibited some experimental studies.

Methods

The β_2 microglobulin clearance and D.D.A.V.P. test were performed according to Van Oort, Monnens and van Munster [3] and Monnens et al [4]. The normal values for the phosphate reabsorption and tubular maximum for phosphate are from Kruse, Kuacht and Göpfert [5]. The acid loading test according to Berg, Aperia and Broberger [6] was done only in the first patient. Human intact parathormone was measured by radioimmunoassay (Immuno Nuclear Corporation, Stillwater, Oklahoma, USA). Human calcitonin was also measured by radioimmunoassay (Immuno Nuclear Corporation).

The renal magensium threshold and Tm Mg werd determined according to Rude, Bethune and Singer [7]. The glomerular filtration rate was measured by the use of inulin. The ultrafiltrable serum magnesium fraction was obtained with the use of Amicon Centrifugo ultrafiltration cones (Amicon, Lexington, Massachusetts, USA).

Serum and urinary magnesium concentrations were determined by atomic absorption spectrophotometry.

In three healthy young adults we found for the magnesium threshold 1.49; 1.25; 1.46 ultrafiltrable-serum-Mg mg/100 ml GFR 1.73 m^2. These values are within the 95% confidence limilts of Rude, Cohen and Singer [8].

Table 1. Renal function in both patients

	M.C.	J.B.
Endogenous clearance of		
creatinine	136-140	122-125
inulin clearance	109	91
ml/min/1.73 m^2 S.A.		
β_2 microglobulin clearance/		
endogenous clearance of		
creatinine	0.5%	-
Phosphate reabsorption	88-92%	87-93%
Tm phosphate		
nmol/min/100 ml GFR	0.98	1.00
Proteinuria	absent	absent
Glucosuria	absent	absent
Abnormal aminoaciduria	absent	absent
D.D.A.V.P.-test		
mOsm/kg	1056	1129
Acid loading test		
according to Berg et al	normal	-
Calciuria	0.13-0.34 mmol/ 24 hr	0.57-0.88 mmol/ 24 hr

Intestinal magnesium absorption studies were made with ^{28}Mg [9]. After fasting overnight 900 kBq (kiloBequerel),(24 μCi)^{28} Mg, dissolved in 0.01 mol HCl, was given

orally to the patient. During seven days, the radioactivity present in the urine and faeces was measured and expressed as percentage of the oral dose. Moreover, in patient MC the total body radioactivity was measured with a shadow-shield Whole Bodycounter. After correction for physical decay and for the background radiation, measured just before the administration of the dose, the retention could be determined. If measurements with the Whole Bodycounter were not performed (JB), the absorbed fraction was calculated as the difference between the administered dose and the amount of radioactivity, found in the feces.
A renal biopsy was taken percutaneously in the second patient and prepared for light microscopy, immunofluorescence and electronmicroscopy with routine techniques.

RESULTS

The renal function of both patients is presented in Table 1. The only abnormality observed with the exception of the magnesium metabolism was a lowered excretion of calcium in the urine. Normally the calcium excretion in children varies between 1.1 and 7.4 mg/kg/24 hours [10].
Almost all family members with a lowered serum magnesium also showed a lowered calcium excretion in the urine. The only person with a low calcium excretion and a normal serum magnesium was an 82 year-old lady (Fig. 3).

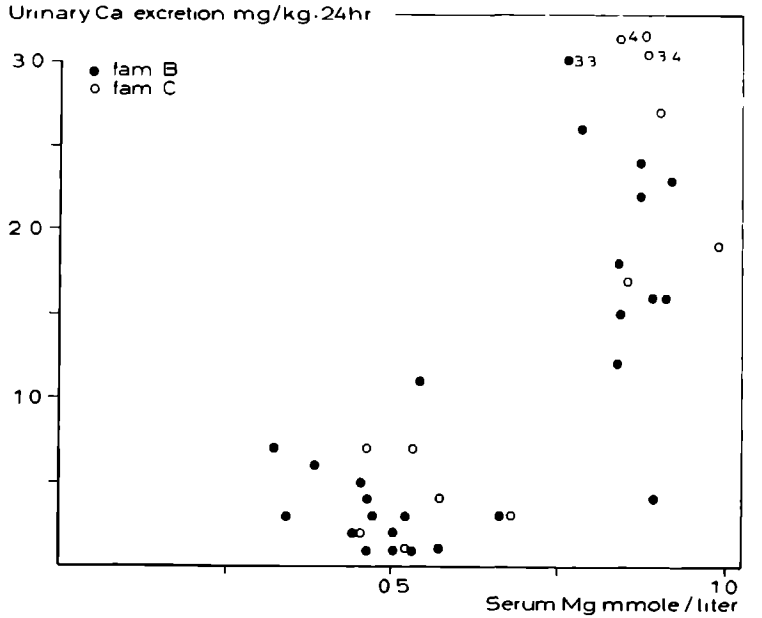

Figure 3. Urinary calcium excretion related to Mg serum level in both families. Symbols are: (•) family B; (o) family C.

Table 2. Serum magnesium and urinary magnesium excretion in both patients

	M.C.	J.B.
Serum magnesium	0.38-0.56 mmol/liter	0.39-0.55 mmol/liter
Urinary excretion of magnesium	8.3 mmol/24 hr	7-9.5 mmol/24 hr

All family members had a normal serum calcium concentration. The values of the serum magnesium and magnesium excretion in the urine of both families are shown in Table 3. Wilcoxon two-sample test showed a highly significant difference ($P<0.001$) in calcium excretion between the normo- and hypomagnesemic persons.

Table 3. Magnesium and calcium levels in serum and urinary excretion of magnesium and calcium in members of both families

	sex	Age	Serum Mg mmol/l	Serum Ca mmol/l	Urinary excretion of Mg mmol/24hr	Ca mmol/24hr
Family C						
II-1	M	48	0.52	2.44	6.9	0.2
II-3	F	44	0.65	2.46	4.1	0.5
II-5	F	37	0.53	2.40	3.1	1.3
III-1	M	20	0.46	2.42	7.8	1.1
III-3	F	13	0.57	2.48	3.5	0.5
Family B						
II-1	F	53	0.47	2.29	8.3	0.4
II-3	F	51	0.32	2.32	5.5	1.1
II-5	F	50	0.35	2.38	4.9	0.9
II-7	F	48	0.52	2.36	5.5	0.3
II-9	F	46	0.46	2.32	4.1	0.6
II-11	F	45	0.46	2.34	6.6	0.2
II-15	M	42	0.38	2.46	6.2	1.3
II-16	M	38	0.34	2.29	5.1	0.6
III-1	F	29	0.32	2.24	-	-
III-9	M	23	0.42	2.48	-	-
III-10	M	22	0.50	2.52	3.9	0.4
III-11	F	21	0.50	2.40	2.5	0.2
III-13	M	23	0.44	2.56	6.9	0.3
III-15	F	20	0.58	2.46	5.6	0.2
III-16	M	13	0.60	2.37	3.6	0.4
III-18	F	17	0.54	2.28	4.2	1.7
III-21	M	9	0.52	2.46	3.0	0.2

For the ^{28}Mg resorption studies, we used exactly the procedure as proposed by Becker, Lombeck and Bremer [9]. In normal adults the total body retention after 72 hours is 20 to 34%, the resorption is 24 to 32%.
Figure 4 shows the results of the oral 28Mg resorption test in M.C. The total body retention was higher than normal as well as the resorption by the gut. J.B. excreted 51.8% of the amount ingested in his feces by 72 hours. Urinary excretion amounted to 13.6% of the administered dose. The calculated retention was 34.6, the calculated resorption of 48.2% was increased.

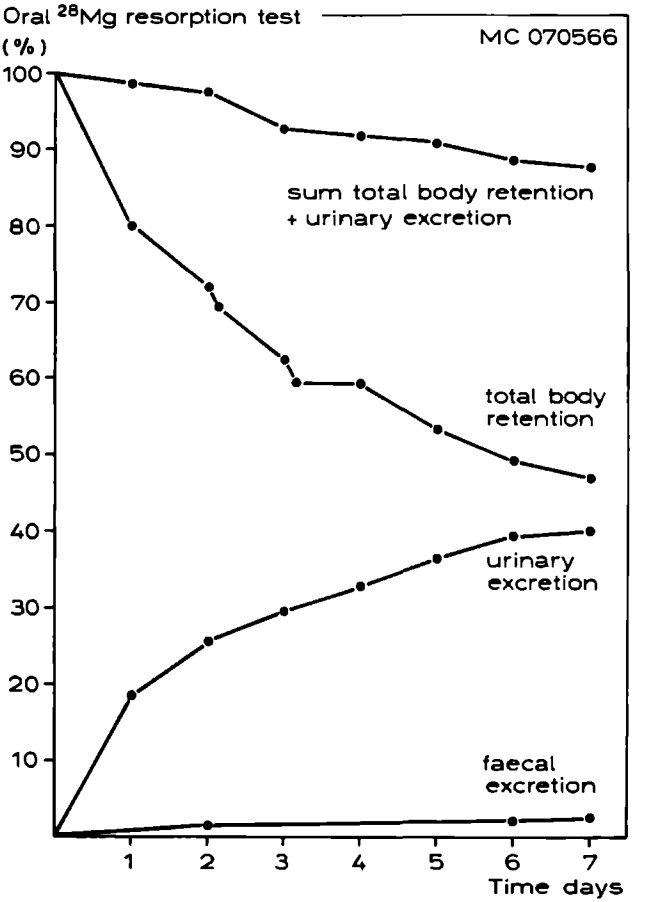

Figure 4. Oral ^{28}Mg resorption test in patient M.C. shows a high resorption rate.

The results of the magnesium infusions are shown in Figure 5. Not only the renal magnesium threshold but also the Tm Mg was lowered in both patients. Triamterene

administration was not able to reduce the urinary magnesium excretion in both patients [11].

Renal biopsy findings in J.B.

Light microscopy and immunofluorexcence study (IgG, IgM, IgA, C_{1q}, C_3, fibrin, albumin) revealed no abnormalities. Under electromicroscopy the glomeruli appeared normal. The thickness and structure of the tubular basement membrane were normal.

Family studies

In the indicated members (see Table 3, Figs. 1 and 2) of both families serum magnesium and urinary excretion of magnesium and calcium were measured. The presence of hypomagnesemia in successive generations and the male to male transmission proves the existence of an autosomal dominant mode of inheritance.

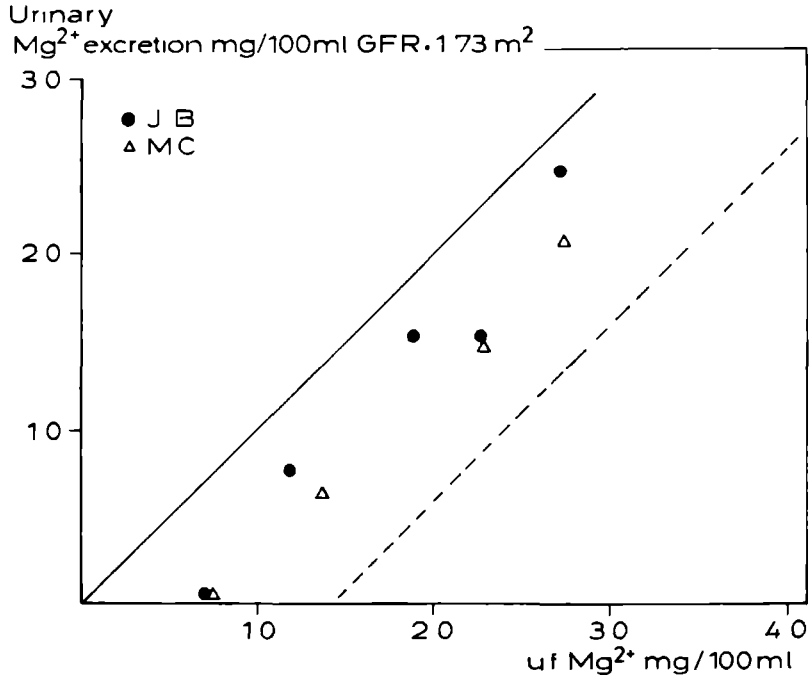

Figure 5. Ultrafiltrable serum magnesium concentrations are plotted against urinary magnesium excretion in patient M.C.(Δ) and J.B.(●) prior to and during magnesium infusions. Urinary excretion along the ordinate is expressed as mg/100ml glomerular filtrate/1.73 m^2 surface area. The concentrations of ultrafiltrable magnesium are presented along the abscissa. The broken line represents the normal regression line according to Rude et al.[7].

DISCUSSION

Disorders causing magnesium deficiency commonly occur in the gastrointestinal tract or the kidney. A defect in the intestinal absorption of magnesium could be excluded in our patients. During magnesium deprivation urinary magnesium excretion can be found to be as low as 0.2 to 0.4 mmol/24 hours [12]. In our patients the urinary excretion of magnesium was in the normal range, although the serum magnesium concentration was distinctly lowered, pointing to a disturbance of tubular reabsorption of magnesium. The different causes of renal magnesium loss causing magnesium deficiency are reviewed by Rude and Singer [13]. Hypercalcemia, primary aldosteronism, the use of drugs such as cis-platinum, osmotic diuresis and a number of different renal diseases causing renal tubular damage could be eliminated as an explanation for renal magnesium loss in our patients.

The absence of clinical symptoms related to Mg deficiency in our two families was striking. Muscular weakness and incoordination, ataxia, vertigo, coarse and irregular tremor were absent. Positive Chvostek's and Trousseau's signs could not be elicited. Frank tetany was never observed. Gastrointestinal symptoms were lacking. The electrocardiogram and electromyogram revealed no abnormalities. The adult patients in the second family (J.B.), however, developed symptoms of pseudogout. This can possibly be related to low values of magnesium and alkaline phosphatase activity in the synovial fluid [14]. The lack of these symptoms in our first family still requires an explanation. In both patients normal levels of intact parathormone were measured. This is reflected in the normal values for Tm phosphate. The influence of chronic hypomagnesemia on parathyroid hormone excretion is confusing. Both lowered and increased levels are reported in human beings [15].

Various micropuncture methods have characterized the tubular handling of magnesium [16]. In the proximal convoluted tubule 20 to 30% of the filtered magnesium. The distal convoluted tubule reclaims only 1 to 5% of filtered magnesium. The loop of Henle has a major role in the reabsorption of magnesium.

The calcium-magnesium interaction located at the basolateral membrane of the loop of Henle allows greater levels of calcium reabsorption when the plasma magnesium concentration is depressed. This speculative model of magnesium transport by the thick ascending limb of Henle's loop is in agreement with the hypocalciuria detected in our patients.

Hereditary isolated renal magnesium loss is, as far as we know, for the first time described in 1966 by Freeman and Pearson [17]. The hypomagnesemia due to urinary loss of magnesium was present in a mother and one of her five children. When serum magnesium varied between 0.45 and 0.50 mmol/liter the calcium excretion varied between 74 and 140 mg/24 hours or 1.6 to 3.1 mg/kg/24 hours. This calcium excretion is higher than measured in the members of our two families with hypomagnesemia. Milazzo et al [18] described also a mother with pseudo-gout and hypomagnesemia. Hypomagnesemia was also present in one of her two children, a 22-year-old son. The calcium excretion amounted to 26 mg/24 hours in the mother and to 52 mg/24 hours in her son, both low values. Information is not available about the hereditary character or the calciuria of the three patients with magnesium depletion secondary to

a renal magnesium leak described by Rude, Cohen and Singer [8]. In contrast to their patients, the renal-tubular maximum for magnesium was lowered in our two patients. This Tm process, however, is a composite of distinct transport properties of the proximal tubule and the loop of Henle as shown by Wong, Dirks and Quamme [19].

ACKNOWLEDGEMENTS

This study is supported by the Dutch Kidney Foundation. Ir. Th. de Boo made the statistical calculations.

REFERENCES

1. Evans RA, Carter JN, George CRP, Walls RS, Newland RC, McConnell GD, Lawrence JR The congenital "Magnesium-losing kidney" report of two patients. Q J Med 197 39-52, 1981
2. Fischer TJWF, Lijnen P, Monnens L, van Munster P, Jansen M, Peer P. Levels of renin, angiotensin I and II, angiotensin converting enzyme and aldosteron ininfancy and childhood Eur J Pediatr 141:3-7, 1983
3. Van Oort A, Monnens L, Van Munster P: Beta-2-microglobulin clearance, an indicator of renal tubular maturation Int J Pediatr Nephrol 1 80-84, 1980
4. Monnens L, Smulders Y, van Lier H, de Boo T. DDAVP test for assessment of renal concentrating capacity in infants and children. Nephron 29:151-154, 1981
5. Kruse K, Kracht U, Göpfert G: Renal threshold phosphate concentration ($TmPO_4$/GFR). Arch Dis Child 57·217-223, 1982
6. Berg U, Aperia A, Broberger O: Subclinical defects in renal regulation of acid-base balance in children with recurrent urinary tract infections. Acta Paediatr Scand 60.521-527, 1971
7. Rude RK, Bethune JR, Singer FR. Renal tubular maximum for magnesium in normal hypoparathyroid and hyperparathyroid man J Clin Endocrinol Metab 51 1425-1431, 1980
8. Rude RK, Cohen BW, Singer FS: Hypomagnesemia of renal origin abnormal renal magnesium threshold and normal Tm Mg Magnesium 2:62-69, 1983
9. Becker K, Lombeck I, Bremer HJ: Primäre hypomagnesiämie Monatschr Kinderheilkd 129.37-42, 1979
10. Paunier L, Borgeaud M, Wijss M: Urinary excretion of magnesium and calcium in normal children. Helv Acta Paediatr 25.577-584, 1970
11. De Vane J, Ryan MP: The effects of amiloride and triamterene on urinary magnesium excretion in conscious saline-loaded rats. Br J Pharmac 72 285-289, 1981
12. Thorén L: Magnesium metabolism. Progress in Surgery 9 131-156, 1971
13. Rude RK, Singer FR. Magnesium deficiency and excess. Ann Rev Med 32·245-259, 1981
14. Cohen L, Kitzes R· Pseudogout with low values of magnesium and low alkaline phosphatase activity in synovial fluid Israel J Med Sci 19.8380840, 1983
15. Massry SG: Pharmacology of magnesium Ann Rev Pharmacol Toxicol 17·67-82, 1977
16. Quamme GA, Dirks JH: Magnesium transport in the nephron Am J Physiol 239.F393-401, 1980
17. Freeman RM, Pearson E: Hypomagnesemia of unknown etiology. Am J Med 41 645-656, 1966
18. Milazzo SC, Ahern MJ, Cleland LG, Henderson DRF: Calcium pyrophosphate dihydrate deposition disease and familial hypomagnesemia. J Rheumatol 8 767-77, 1981
19. Wong NLM, Dirks JH, Quamme GA: Tubular reabsorptive capacity for magnesium in the dog kidny. Am J Physiol 244.F78-F83, 1983

ISOLATED AUTOSOMAL RECESSIVE RENAL MAGNESIUM LOSS IN TWO SISTERS

Published in: Clinical Genetics 1987; 32: 398-402.

ISOLATED AUTOSOMAL RECESSIVE RENAL MAGNESIUM LOSS IN TWO SISTERS

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SUMMARY

A familial autosomal recessive form of isolated renal magnesium loss is presented. Two children in this family suffered from convulsions unrelated to hypomagnesemia. Magnesium infusion studies revealed a lowered threshold but a normal tubular maximum for magnesium.
In contrast to two families with the autosomal dominant form of isolated renal magnesium wasting, the calcium excretion in the urine was normal.

INTRODUCTION

Magnesium depletion due to congenital impairment in tubular reabsorption is uncommon. Mostly it is part of a more extensive tubular disturbance (Evans et al. 1981). Isolated magnesium loss by the kidney appears to be extremely rare. Recently we reported two unrelated families with renal magnesium loss with autosomal dominant inheritance (Geven et al. 1987). In this study we present two sisters with isolated magnesium wasting. The inheritance is of the autosomal recessive type.

PATIENTS AND METHODS

V.v.d. E (patient 1) is the fourth of four children. She was evaluated for moderate psychomotor retardation and convulsions at 4 years of age. Uneventfull birth took place after a normal pregnancy. The first generalised convulsion occurred at the age of three months. Neurologic investigation showed delayed speech and linguistic development. The electroencephalogram presented no epileptic activity, while a CT-scan showed a mild to moderate central and cortical atrophy of both hemispheres. Therapy with valproate was started.
Serum magnesium was 0.56 mmol/l (normal 0.75-1.20 mmol/l). Laboratory data revealed a normal serum sodium 138 to 143 meq/l, normal serum potassium 4.0 to 4.5 meq/l, normal serum choride 105 meq/l, normal serum bicarbonate 26.0 mmol/l, normal blood pH 7.40, normal serum calcium 2.32 to 2.48 mmol/l and a normal ionized calcium (1.30 mmol/l). Plasma renin activity and plasma aldosterone concentration were in the normal range. Parathormone level was 5.8 pmol/l (normal <10). Calcitonin concentracion was 11 pmol/ml (normal < 28 pmol/ml). The intravenous urogram showed no abnormalities.

Table 1. Serum magnesium and urinary magnesium excretion in both patients.

	patient one	patient two
serum magnesium	0.53-0.66 mmol/l	0.56-0.62 mmol/l
urinary excretion of magnesium	2.9-3.7 mmol/24 hr	3.9-6.6 mmol/24hr

The renal function and magnesium metabolism were more extensively investigated in this girl (tables 1 and 2).

S.v.d. E. (patient 2), a sister of patient 1, and 4 years older than her, was born at term after prolonged bedrest of the mother during pregnancy because of cervix insufficiency. Delivery was normal. In the first year convulsions were observed during febrile infectious periods. Therapy was started with phenobarbital and afterwards valproate was given. She showed mild psychomotor retardation. Neurologic investigation revealed no abnormalities except brisk tendon reflexes. Electroencephalogram showed an epileptic pattern after photostimulation. The CT-scan of the brain was normal. Laboratory investigations presented a low serum magnesium of 0.53 mmol/l (normal 0.75 to 1.20 mmol/l), normal serum sodium 140 to 141 meq/l, normal serum potassium 4.0 to 4.2 meg/l, normal serum bicarbonate 26.0 mmol/l, normal blood pH 7.37, normal serum calcium 2.50 mmol/l and normal ionized calcium 1.30 mmol/l. Plasma parathormone level (7.0 pmol/l) and plasma calcitonin (16 pmol/ml) were in the normal range.

Table 2. Renal function in both patients.

	patient one	patient two
endogeneous clearance of creatinine	86-157	107-136
inulin clearance ml/min/1.73 m^2 S.A.	110-114	84
B_2 microglobulin clearance/ endogeneous creatinine clearance	0.04%	--
phosphate reabsorption	85-95%	85-87%
Tm phosphate mmol/min/100ml GFR	1.1	1.2
proteinuria	absent	absent
glucosuria	absent	absent
abnormal aminoaciduria	absent	absent
D.D.A.V.P. test mOsmol/kg	1056	--
calciuria mg/kg/24 hr	2.1-3.4	1.9-2.0

The B_2 microglobulin clearance and D.D.A.V.P. test were performed according to van Oort et al. (1980) and Monnens et al. (1981). The normal values for the phosphate reabsorption and tubular maximum for phosphate are from Kruse et al. (1982). Human intact parathormone was measured by radioimmunoassy (Immuno Nuclear Corporation, Stillwater, Oklahoma, USA). The magnesium threshold and Tm Mg were determined according to Rude et al. (1980). The glomerular filtration rate was measured by use of inulin. The ultrafiltrable magnesium fraction was obtained with the use of MPS-1 system (Amicon, Danver, Massachustts, USA). Serum and urine magnesium concentrations were determined by atomic absorption spectrophotometry.

In three healthy young adults we found for the magnesium threshold: 1.49; 1.25; 1.46

ultrafiltrable Mg mg/100ml and the TmMg to be: 1.55; 1.10; and 1.15 mgMg/100ml GFR. 1.73m^2. These values are within the 95% confidence limits of Rude et al. (1980).

Intestinal magnesium absorption studies were performed with ^{28}Mg.(Becker et al. 1979). After an overnight fast 481 kBq (kiloBequerel),(=13μCi) ^{28}Mg, dissolved in 0.01 mmol HCl, was given to the patient orally. For 6 days, the radioactivity present in feces and urine was measured and expressed as percentage of the oral dose. At the same time the total body radioactivity was measured with a shadow-shield Whole Bodycounter. After correction for physical decay and for the background radiation, measured just before the administration of the dose, the retention could be determined.

RESULTS

Table 1 summarises serum magnesium levels and urinary excretion in the two sisters. The renal function of both patient is presented in table 2. All function studies revealed normal values including the calcium excretion in the urine. In relation to the low serum magnesium, the magnesium excretion in the urine was clearly too high.

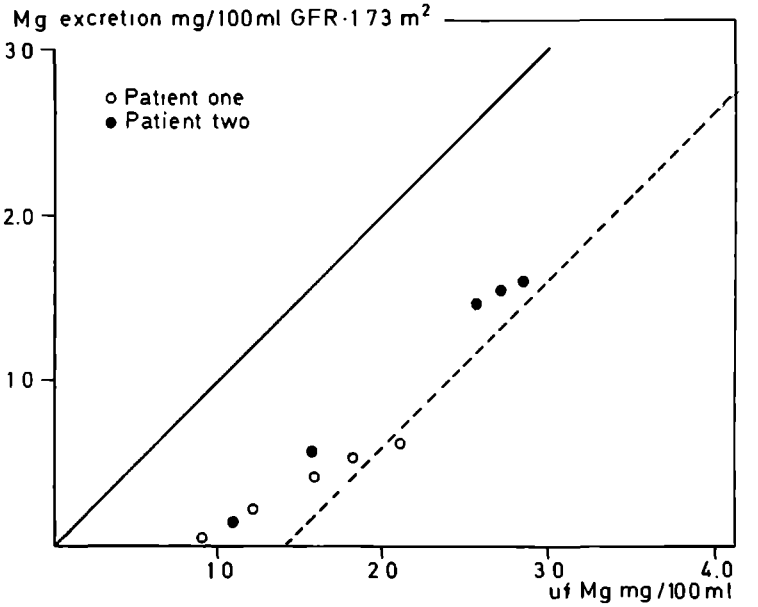

Figure 1. Ultraflitrable serum magnesium concentrations are plotted against urinary magnesium excretion in Patient 1 (o) and patient two (•) prior to and during magnesium infusions. Urinary magnesium excretion along the ordinate is expressed as mg/100 ml glomerular filtrate/1.73 m^2 surface area. The concentrations of ultrafiltrable magnesium are presented along the abcissa. The broken line represents the normal regression line according to Rude et al (1980).

Figure 1 shows the results of the magnesium infusions. The renal magnesium threshold was lowered (0.91 and 1.08 mg uf-Mg/100 ml, respectively) in both patients, while the TmMg appeared to be normal.For the ^{28}Mg resorption studies, we used exactly the procedure proposed by Becker et al (1979). In normal adults the total body retention after 72 hours is 20 to 34%, the resorption is 24 to 32%. Figure 2 presents the results of the oral ^{28}Mg resorption test in Patient 1. The total body retention and resorption by the gut were higher than normal.

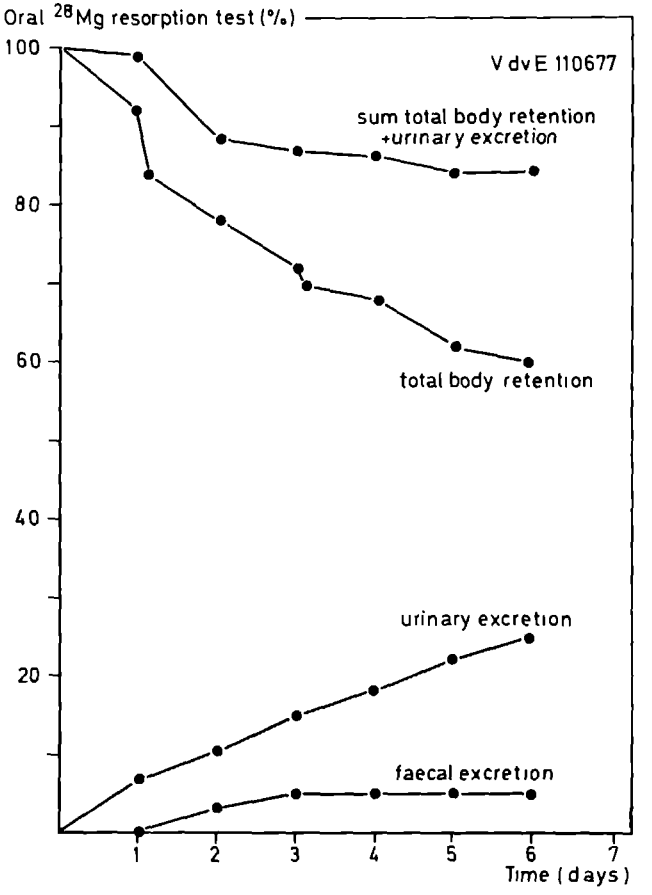

Figure 2. Oral ^{28}Mg resorption test in Patient 1 shows a high resorption rate.

Family studies

In the family members indicated (fig. 3), serum magnesium, serum sodium, serum

potassium and serum calcium were measured and were all in the normal range. Besides the parents, who are second cousins, another couple who are second cousins is present in this family; their two children had normal psychomotor development, with no convulsions and no abnormalities observed in the laboratory investigations. Based on the normal results of the parents, the occurence in sibs, and the consanguinity, we presume an autosomal recessive mode of inheritance for the isolated renal magnesium loss.

DISCUSSION

Commonly magnesium deficiency is caused by disorders affecting the gastrointestinal tract or the kidney. A defect in the intestinal absorption of magnesium could be excluded in Patient 1. Urinary magnesium excretion can be found as low as 0.2 to 0.4 mmol/24 hours during magnesium deprivation (Thorèn 1971).

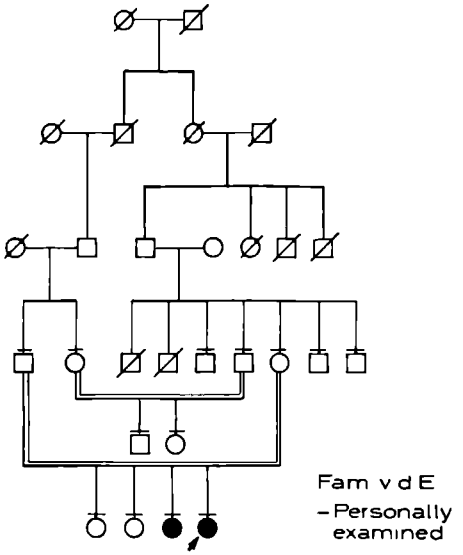

Figure 3. Pedigree of the family. Arrow (↗) indicates Patient 1; (–) indicates persons personally examined. Two pairs of second cousins are present and an autosomal recessive mode of inheritance is found.

In these two patients the urinary magnesium excretion was in the normal range, although the serum magnesium level was lowered, pointing to a disturbance of tubular reabsorption of magnesium. Rude and Singer (1981) reviewed the different causes of renal magnesium wasting causing magnesium deficiency. Hypercalcemia, primary aldosteronism, the use of drugs such as cis-platinum, osmotic diuresis and a number of

different renal diseases causing renal tubular damage could be eliminatedas an explanation for the renal magnesium loss in our patients. Except for the brisk tendon reflexes, no clinical symptoms possibly related to magnesium deficiency were found. Positive Chvostek's and Trousseau's sign were absent, as were muscular weakness and incoordination, ataxia, coarse and irregular tremor. ECG and EMG were normal. In both patients normal values were measured for parathormone, reflected in the normal Tm phosphate. Freeman and Pearson (1966); Milazzo et al. (1981) and Geven et al. (1987) have reported isolated renal magnesium loss. None of these reports showed serum electrolyte abnormalities apart from hypomagnesemia. An autosomal dominant mode of inheritance was demonstrated by Geven et al. (1987). The calcium excretion in the different members of these families with hypomagnesemia was lowered.
In the patients reported here the serum magnesium levels were not as low as in the families with the autosomal dominant form.
In autosomal dominant magnesium loss, not only the renal magnesium threshold was lowered but also the TmMg. The hypothesis was proposed that the hypomagnesemia increased the calcium reabsorption in Henle's loop. The only slightly lowered magnesium level present in the autosomal recessive form apparently had less effect on Henle's loop in relation to the calcium reabsorption.

ACKNOWLEDGEMENTS

This study is supported by a grant from the Dutch Kidney Foundation.

REFERENCES

1 Becker, K., I. Lombeck, & H J Bremer (1979). Primäre Hypomagnesiämie Monatschr Kinderheilkd 129, 37-42.
2. Evans, R A., J N Carter, C R P. George, R S Walls, R.C. Newland, G D McConnel & J R Lawrence (1981) The congenital "Magnesium-loosing kidney" report of two patients Q J Med. 197, 39-52
3. Fiselier, T.J.W F., P. Lijnen, L.A.H. Monnens, P. Van Munster, M Jansen, & P Peer (1983) Levels of renin, angiotensin I and II, angiotensinconverting enzyme and aldosteron in infancy and childhood Eur J. Pediatr. 141, 3-7
4. Freeman, R M. & E. Pearson (1966) Hypomagnesemia of unknown etiology Am J Med 41, 645-655.
5. Geven, W.B, L.A.H. Monnens, J L. Willems, W. Buijs & B G Ter Haar (1987) Renal magnesium wasting in two families with autosomal dominant inheritance Kidney Int 31, 1140-1144.
6. Hedemann, L., P. Strunge & V. Munck (1986). The familial magnesium-losing kidney Acta Med. Scand. 219, 133-136.
7. Kruse, K., U. Kracht & G. Göpfert (1982). Renal threshold phosphate concentration(Tm PO_4/GFR). Arch Dis. Child. 57 217-223.
8. Massry, S.G (1977) Pharmacology of magnesium. Ann. Rev. Pharmacol Toxicol 17, 67-82
9. Milazzo, S.C., M.J. Ahern, L.G. Cleland & D.R.F. Henderson (1981) Calcium pyrophosphate dihydrate deposition disease and familial hypomagnesemia J. Rheumatol. 8, 767-771
10. Monnens, L., Y. Smulders, H. Van Lier & T. De Boo (1981). DDAVPtest for assessment of renal concentrating capacity in infants and children. Nephron. 29, 151-154.

11. Paunier, L., D. Borgeaud & M. Wijss (1970). Urinary excretion of magnesium and calcium in normal children. Helv. Acta. Paediatr. 25, 577-584.
12. Quamme, G.A. & J.H. Dirks (1980). Magnesium transport in the nephron. Am. J. Physiol. 239, F393-F401.
13. Rude, R.K., J.R. Bethune & F.R. Singer (1980). Renal tubular maximum for magnesium in normal, hypoparathyroid and hyperthyroid man. J. Clin. Endocrinol. Metab. 51, 1425-1431.
14. Rude, R.K. & F.R. Singer (1981). Magnesium deficiency and excess. Ann. Rev. Med. 32, 245-259.
15. Rude, R.K., B.W. Cohan & F.R. Singer (1983). Hypomagnesemia of renal origin: abnormal renal magnesium threshold and normal TmMg. Magnesium. 2, 62-69.
16. Thorèn, L. (1971). Magnesium metabolism. Progress in Surgery. 9, 31-156.
17. Van Oort, A., L. Monnens & P. Van Munster (1980). Beta-2-microglobulin clearance an indicator of renal tubular maturation. Int. J. Pediatr. Nephrol. 1, 80-84.
18. Wong, N.L.M., J.H. Dirks & G.A. Quamme (1983). Tubular reabsorptive capacity for magnesium in the dog kidney. Am. J. Physiol. 244, F78-F83.

RENAL PROSTAGLANDIN EXCRETION IN PATIENTS WITH ISOLATED HYPOMAGNESEMIA

Published in: Magnesium-Bulletin 1989; 11: 64-67.

RENAL PROSTAGLANDIN EXCRETION IN PATIENTS WITH ISOLATED HYPOMAGNESEMIA

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SUMMARY

The influence of magnesium depletion in humans with isolated renal magnesium loss, was studied on the renal excretion of prostaglandin E_2, prostaglandin $F_{2\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2. In animal experiments magnesium depletion caused an increased outflow of prostaglandins from the mesenteric arterial bed. In our group of magnesium depleted humans there was no increased formation of prostaglandin E_2, prostaglandin $F_{2\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 in the kidney as reflected by their normal excretion in the urine.

INTRODUCTION

Magnesium is the fourth most abundant cation in the human body and the second most common in the intracellular fluid. Potassium is the most important cation in the intracellular fluid. Potassium depletion in dogs and human beings stimulates renal prostaglandin E synthesis [1]. In rats with experimental magnesium deficiency the plasma and tissue levels of PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2 were significantly higher than in normals [2]. Recently we studied two families presenting with hypomagnesemia due to isolated renal magnesium loss [3]. This offered us the opportunity to evaluate the influence of magnesium depletion on the renal excretion of prostaglandins in human beings.

Table 2. Normal renal prostaglandin and magnesium excretion during hypomagnesemia.

patient	sex	Prostaglandin excretion $PGF_{2\alpha}$ nmol/24h	PGE_2 nmol/24h	TXB_2 nmol/24h	6-keto-$PGF_{2\alpha}$ nmol/24h	Magnesium excr. mmol/24h
1	F	7.90	1.40	1.20	7.10	4.7
2	M	10.00	0.79	0.84	4.60	2.1
3	F	5.70	0.11	1.60	6.60	1.7
4	M	18.00	0.60	1.20	10.00	4.9
5	F	6.00	0.17	1.40	6.80	4.0
6	F	9.20	0.16	0.93	9.80	4.0
7	F	7.80	0.20	1.10	4.80	3.6
8	F	7.20	0.33	1.60	9.70	6.0
9	M	22.00	0.84	2.20	12.00	5.0
10	F	8.10	0.63	1.80	7.80	5.8
11	F	10.00	0.28	2.00	7.80	5.7
12	M	6.70	0.35	1.00	6.10	3.2
Normal values	M	7.6-33.0	0.40-4.8	0.86-5.3	3.0-8.7	2.5-
M n = 20	F	3.6-	0.07-	0.70-	2.2-	5.0
F n = 18		10.0	0.98	2.3	11.0	[19]

MATERIALS AND METHODS

Twelve patients with hypomagnesemia due to isolated renal magnesium loss could be studied. In each of them analysis of serum and urinary magnesium and also the magnesium content of mononuclear cells (mo-Mg) was performed according to methods described earlier [4].The urinary excretion per 24 hour was measured of prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TXB_2) in all patients and compared with control values.

24-hour urine samples were collected and stored at -20° C until assay for prostaglandins. The excretion of PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2 in the urine specimen samples were determined by highly specific radioimmunoassays (RIA) according to the procedures previously described [5,6]. In brief, the assays include the following steps: to 1 ml urine samples an amount of 17 Bq PG tracer {5,6,11,12,14,15(n)-^{3}H} $PGF_{2\alpha}$ sp.act. 5.92 TBq/mmol, Amersham International plc (U.K.) was added, since it could be demonstrated that the addition of a single titrated PG was sufficient to monitor the similar procedural losses of the four PGs throughout the next steps. The samples were acidified with 0.1 ml 1 n HCl to pH 3.5, extracted with 2x10 ml ethyl acetate and the solvent residue was applied to gel filtration on Sephadex G-25. An aqueous, low molecular mass effluent was collected containing the PGs with recoveries of {^{3}H}$PGF_{2\alpha}$ between 50 and 70%. The effluent selected was applied to specific radioimmunoassay for $PGF_{2\alpha}$, PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 and the results obtained were corrected for percentage recovery. Cross-reactivity at 50% of {^{3}H} PG displacement for the anti-$PGF_{2\alpha}$ antiserum (obtained from Dr. H.R.Behrman, Harvard Medical School Boston, Mass., U.S.A.) was 17% with $PGF_{1\alpha}$. The anti-PGE_2 antiserum (purchased from Institute Pasteur Production, Paris, France) showed 10.5% cross-reaction with PGE_1 . The anti-6-keto-$PGF_{1\alpha}$ antiserum (purchased from Seragen Inc., Boston, Mass., U.S.A.) cross-reacted 12.8% with PGE_1 , 1.4% with PGE_2 and 7.4% with $PGF_{2\alpha}$. The other PGs tested (E,F,A,B) gave values of < 0.1% with the antiserum used, including the one directed towards TXB_2 (obtained from Dr. F.A.Fitzpatrick, The Upjohn Company, Kalamazoo, Mich., U.S.A.). The PGE_2 assay used {5,6,8,11,12,14,15(n)-^{3}H} PGE_2 (Amersham International plc, Amersham, U.K.) with a sp.act. of 5.92 TBq/mmol. The tritium labeled preparations of the 6-keto-$PGF_{1\alpha}$ and the TXB_2 assays were 6-keto-{5,6,8,9,11,12,14,15-(n)-^{3}H}-$PGF_{1\alpha}$ and {5,6,8,9,11,12,14,15-(n)-^{3}H}-TXB_2 (sp.act. 6.04 TBq/mmol), respectively, and were purshased from New England Nuclear Boston, Mass., U.S.A.

Sensitivities of the four assays as defined at B/B_0 =0.9 following logit-log transformation of the assay data, were 1.4 fmol PGE_2 , 8.5 fmol $PGF_{2\alpha}$, 10.0 fmol 6-keto-$PGF_{1\alpha}$ and 8.4 fmol TXB_2 per assay tube. Method blanks were below the sensitivity limits. Intra and interassay variabilities were calculated from a urine pool stored at -70° C. After ten concecutive assays the PGE_2 determinations showed 6.4% and 15%, the $PGF_{2\alpha}$ assay 5.3% and 11%, the 6-keto-$PGF_{1\alpha}$ assay 7.3% and 9.6% and the TXB_2 assay 8.1% and 12.5% intra- and interassay variability, respectively.

Table 1. Values of serum magnesium, erythrocyte magnesium and mononuclear magnesium in the patients with isolated renal magnesium loss. In all patients 3 repeated determinations were performed.

Patient	age	sex	se-Mg mmol/l			er-Mg µg/gr RBC			mo-Mg µg/cell			mo-Mg µg/mg prot.			mo-Mg µg/mg DNA		
1	50	F	0.34	0.50	0.36	113	126	122	125	93	91	1.18	1.34	1.49	13.1	10.6	16.9
2	27	M	0.39	0.46	0.41	110	115	155	89	80	57	1.05	1.31	0.87	9.2	9.1	9.2
3	24	F	0.44	0.51	0.52	126	132	127	95	78	89	1.04	1.29	1.02	10.5	9.9	10.8
4	17	M	0.61	0.61	0.65	130	139	128	68	92	62	0.96	1.18	0.88	7.5	8.7	7.3
5	49	F	0.56	0.44	0.50	120	114	110	97	73	85	0.98	0.92	0.86	10.3	8.0	13.7
6	25	F	0.44	0.44	0.43	125	125	122	106	120	117	1.34	1.22	1.32	10.6	12.1	15.7
7	52	F	0.43	0.44	0.59	144	133	136	92	102	89	1.29	1.10	1.19	9.9	10.9	11.9
8	57	F	0.55	0.56	0.52	151	149	143	104	102	109	1.19	1.04	1.15	12.7	10.6	14.7
9	42	M	0.35	0.46	0.41	118	110	119	112	98	82	1.22	1.12	0.92	11.7	9.0	10.5
10	55	F	0.41	0.35	0.37	102	99	101	82	87	69	1.15	1.21	0.94	9.0	9.5	9.0
11	54	F	0.46	0.52	0.43	126	122	125	102	86	51	1.34	1.25	0.65	11.3	8.8	6.5
12	27	M	0.32	0.31	0.35	87	85	85	81	88	91	1.10	1.15	1.01	9.3	9.4	11.6
mean			0.44	0.47	0.46	121	121	123	96	92	83	1.15	1.18	1.03	10.4	9.7	11.5
sd			0.09	0.08	0.09	17	18	18	15	13	20	0.13	0.12	0.23	1.6	1.1	3.3
Normal values			0.75 - 1.00			125 - 191 n = 56			49 - 148 n = 56			0.75 - 1.34 n = 56			6.5 - 18.3 n = 56		

Statistical methods

Wilcoxon's signed rank test was used for detection of differences between determined values and Spearman's rank correlation coefficients to detect a possible relationship between serum and erythrocyte magnesium content. Results were considered to be significant when $p<0.05$.

RESULTS

As shown in table 1 all patients had lowered levels of serum magnesium. Serum concentrations of sodium, potassium, calcium, phosphate, creatinine, urea, and PTH were within normal ranges in all patients. Mean er-Mg was decreased compared to the control values ($p< 0.01$). These control values were obtained from 56 normal adults (females and males showed no significant difference between both sexes).
Figure 1 shows all se-Mg and corresponding er-Mg values. There was a good correlation between se-Mg and er-Mg ($r=0.79$, $p=0.002$). The mean content of mononuclear magnesium expressed as μg Mg/mg protein was significantly lower than

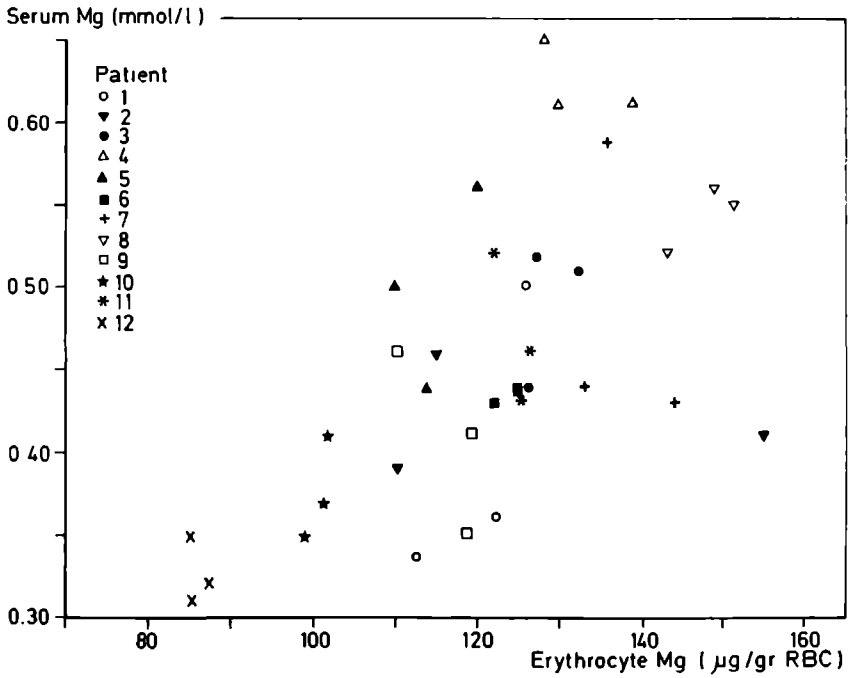

Figure 1: Serum-magnesium levels with corresponding erythrocyte magnesium values show a good correlation ($r=0.79$, $p=0.002$).

control values ($p<0.05$). No lowered mo-Mg was found when the magnesium content of mononuclear cells was expressed as fg Mg/cell and μg Mg/mg DNA. Although the serum magnesium concentration was decreased, the urinary excretion of magnesium was within the normal range pointing to a renal reabsorption defect for magnesium. In these magnesium depleted patients the renal excretion of the four measured prostaglandins, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2, was in the normal adult range (table 2).

DISCUSSION

It is obvious that potassium depletion in the dog, in the rabbit and also in the human stimulates the renal PGE_2 synthesis [7]. So it was tempting to study the influence of depletion of magnesium, as the second most intra-cellular cation in the human, on the renal prostaglandin excretion. All our patients had distinctly lowered serum magnesium concentrations. As was already observed in the dogs [4] erythrocyte magnesium content appears to reflect better the body magnesium stores than mononuclear magnesium levels. In our patients a normal value of urinary prostaglandin excretion was demonstrated. This is in contrast to the experimental data of Nigam et al [2]. They measured the plasma and tissue concentrations of prostanoids PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_1\alpha$ and TXB_2 in normal rats and in rats receiving a magnesium deficient diet for 12 weeks. The mean values of the plasma and tissue levels of the four prostanoids were significantly higher in the magnesium deficient rats. Their approach, however, can be criticized. Plasma and tissue levels of prostanoids are considered not to be meaningful as indices of in vivo product formation [8,9,10]. The primary compounds have very short biological half-life and the possibility of ex-vivo formations by a varity of stimuli is great. The invasive sampling via a catheter already results in an artifactual release of PGI_2 and TXB_2 [8,11]. Another explanation for the discrepancy between their and our results could be the species difference and different degree or duration of the magnesium deficiency. In rats on a low magnesium diet the PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 outflow from perfused isolated mesenteric arterial bed was significantly increased compared to controls [12]. These results point in the same direction as these reported by Nigam et al. [2]. The assumption has been made that the urinary excretion of the four prostaglandins studied, reflects mainly renal biosynthesis. No definite conclusions however, have been reached. The prostaglandins PGE_2 and $PGF_{2\alpha}$ have been the most extensively studied. The renal medullary interstitial cells and collecting tubles are the major sites of PGE_2 and $PGF_{2\alpha}$ synthesis. After intravenous injection in man no intact labelled PGE_2 could be recovered from the urine [13]. Following their synthesis PGE_2 and $PGF_{2\alpha}$ can be subjected to metabolism [14] and transport to the venous circulation [15]. In addition the urinary excretion is influenced by urinary pH [16,17] and flowrate [16,17,18]. With these restrictions in mind we were unable to demonstrate an effect of magnesium depletion on the renal biosynthesis of prostaglandins in our group of patients with magnesium deficiency.

ACKNOWLEDGEMENTS

We thank Th. de Boo and W. Lemmens from the department of statistical consultation for their help. This study was supported by the Dutch Kidney Foundation.

REFERENCES

1. Senba, S., K.Konishi, T.Saruta, Y.Ozawa, E.Kato, Y.Amagasaki, I.Nakata: Hypokalemia and prostaglandin overproduction in Bartter's syndrome. Nephron **37** (1984) 257-263.
2. Nigam, S., R.Averdonk, T.Gunther: Alteration of prostanoid metabolism in rats with magnesium deficiency. Prostagl Leuk. Med. **23** (1986) 1-10.
3. Geven, W.B , L.Monnens, J.Willems, W Buijs, B.Ter Haar Renal magnesium wasting in two families with autosomal dominant inheritance Kidney Int. **31** (1987) 1140-1144
4. Geven, W B., G M.Vogels-Mentink, J.L.Willems, Th de Boo, W Lemmens, L A H Monnens Experimental magnesium depletion in the dog. Influence on the magnesium content of mononuclear cells, erythrocytes, muscle and bone. Mag Bull. **10** (1988) 45-50
5. Thomas, C M.G., R.J van den Berg, H J de Koning Gans et al · Radioimmunoassay for prostaglandins I. Technical validation of prostaglandin F_{2a} measurements in human plasma using sephadex G-25 gelfiltration. Prostaglandins **15** (1978) 839-849.
6. Thomas, C M G , R J van den Berg, H J.de Koning Gans et al. Radioimmunoassay II Measurement of prostaglandin E_2 and 13,14-dihydro-15-keto metabolites of the E and F series. Description of a reliable technique with universal applicability Prostaglandins **15** (1978) 849-855.
7. Ferris, T.F.: Prostaglandins, potassium and Bartter syndrome. J. Lab. Clin. Med **92** (1978) 663-668.
8. Catella, F., J Nowak, G.A.Fitzgerald: Measurement of renal and non-renal eicosanoid synthesis. Amer. J. Med. **81** (1986) 23-29.
9. Seyberth, H.W., P.G.Kuhl: The role of eicosanoids in pediatrics. Eur J Pediatr. **147** (1988) 341-349.
10. Vesterqvist, O.: Measurements of the in vivo synthesis of thromboxane and prostacyclin in humans. Scand. J. Clin. Lab. Invest. **48** (1988) 401-407.
11. Fitzgerald, G.A., A.K.Pedersen, C. Patrono. Analysis of prostacyclin and thromboxane biosynthesis in cardiovascular disease. Circulation **67** (1983) 1174-1177
12. Soma, M., S.C.Cunnane, D.F.Horrobin, M.S.Manku, M Honda, M Hatano Effects of low magnesium diet on the vascular prostaglandin and fatty acid metabolism in rats Prostaglandins **36** (1988) 431-441.
13. Granstrom, G.: On the metabolism of prostaglandin E_2 in man. Prog biochem Pharmacol **3** (1967) 89-93.
14. Bito, L.Z.: The dependency of renal PG excretion and/or metabolism on facilitated transport processes. Prostaglandins **11** (1976) 472-473.
15. Kauker, M.L.: Tracer microinjection studies of prostaglandin E_2 transport in the rat nephron. J.Pharmacol. Exp. Ther. **193** (1975) 274-280.
16. Haylor, J., C.J.Lote, A.Thewles: The effect of sodium bicarbonate on the flow dependency of urinary prostaglandin excretion in man. Clin. Sci. **70** (1986) 141-145.
17. Filep, J., E.Foldes-Filep: Effect of urinary pH and urinary flow rate on prostaglandin E_2 and kallikrein excretion by the conscious dog. J. Physiol. **383** (1987) 1-8.
18. Roberts, D.G., R.J.Strife, J.G.Gerber et al.: Effect of sustained water diuresis on prostaglandin E_2 excretion in humans. Am. J. Physiol. **284** (1985) F830-F834
19. Parfitt, A.M., M.Kleerekoper: Clinical disorders of calcium phosphorus and magnesium metabolism. In: Maxwell, M.H., C.R. Kleeman, (eds): Clinical disorders of fluid and electrolyte metabolism. McGraw-Hill, New York 1980, 947-1151.

REFERENCE VALUES OF MAGNESIUM AND POTASSIUM IN MONONUCLEAR CELLS AND ERYTHROCYTES OF CHILDREN

Published in: Clinical Chemistry 1990; 36: 1323-1327.

REFERENCE VALUES OF MAGNESIUM AND POTASSIUM IN MONONUCLEAR CELLS AND ERYTHROCYTES OF CHILDREN

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SUMMARY

Reference values for magnesium and potassium contents of mononuclear cells and erythrocytes were estimated in cord blood and in children form infancy through adolesence. No differences were detected between results for boys and girls. The mononuclear magnesium content was independent of age and was within the adult range of values. No significant correlation was shown between magnesium in serum and in mononuclear cells. Mononuclear potassium also showed no age-related differences. The correlation between magnesium and potassium contents in mononuclear cells was significant; however, the correlation was lower when the magnesium and potassium contents were expressed in terms of protein content: micromoles or millimoles per gram of protein, respectively. The concentration of magnesium in erythrocytes was significantly lower in cord blood and during the first month of life, compared with that at older ages, and showed no significant correlation with serum magnesium. The concentration of erythrocyte potassium was independent of age and showed a low but significant correlation with erythrocyte magnesium content.

INTRODUCTION

Magnesium and potassium are the main intracellular cations, with only small percentages of their total amounts in the body being located in extracellular fluid.
After Baron and Ahmed [1] advocated the use of leukocytes for measurements of intracellular ions, an increasing number of papers have proposed the use of magnesium content of mononuclear cells as an index of intracellular magnesium [2].

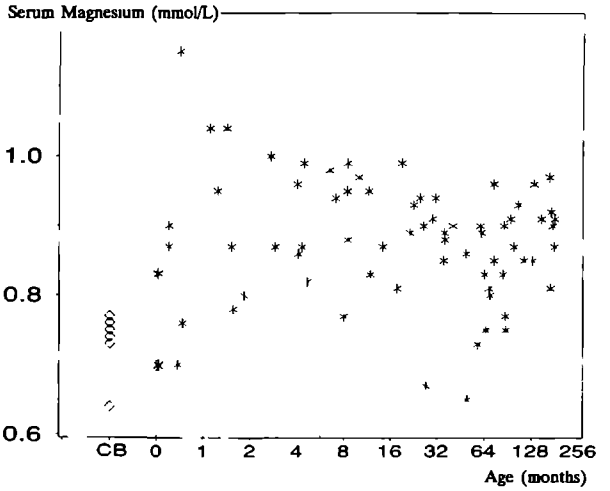

Figure 1. Serum magnesium versus age. CB is cord blood.

Paunier et al. [3], investigating the fetal-maternal relationship of intra- and extracellular magnesium and potassium concentrations, found a significant lower magnesium concentration in erythrocytes of cord blood in comparison with the maternal erythrocytes. The potassium concentration in cord blood erythrocytes, however, was higher than in maternal erythrocytes. The good correlation of magnesium and potassium in lymphocytes and erythrocytes in both cord and maternal blood showed remarkable similarity. But Abraham and co-workers [4] reported the absence of a significant correlation of magnesium and potassium in lymphocytes and erythrocytes.

The aim of this study was to establish reference values of magnesium and potassium in erythrocytes and mononuclear cells in comparison with serum levels in childhood from cord blood through adolescence. Investigation into the possible age-dependancy of intracellular magnesium and potassium values shows considerable gaps. Without reference values, individual data obtained in children with suspected magnesium deficiency or magnesium loosing nephropathy cannot be put into context. This study also supplies new data to the relation of magnesium and potassium in mononuclear cells and erythrocytes.

MATERIALS AND METHODS

Samples approximately 5 - 10 ml of heparinised blood were taken during venipuncture from subjects whose conditions had no known relation to hypomagnesemia, lowered concentrations of potassium in serum or magnesium- or potassium deficiency. Most of the patients in the younger age groups (I and II) were hospitalized; the older ones (Groups III and IV) were mostly outpatients. Previously measured concentrations of serum potassium and magnesiumin these subjects in Group III and IV were within the normal ranges. All patients had normal oral feeding. Ten milliliter cordblood was taken from neonates born vaginally after uneventful pregnancies and births.

We grouped the samples as follows: Group I, 8 samples of cordblood; Group II, 8 samples from the first month postpartum; Group III, 15 samples from ages between 1 and 6 months; and Group IV, 57 samples from ages six to 200 months (6-12 months, eight samples; 12-36 months, 12 samples; 36-72 months, 13 samples; 72-120 months, 10 samples; 120-200 months, 14 samples). Every child in this study was analysed once. All groups contained as many boys as girls, or differed by no more then one in groups with an unequal number of samples. Thus we could evaluate the influence of age and sex on the reference values.

Laboratory methods

Magnesium was measured by atomic absorption spectrophotometry (Perkin Elmer 5000, Norwalk CT). Other measurements were done by routine laboratory methods. In 17 samples, taken at random, the total amount of leukocytes in the venous blood was counted (Coulter Counter, Coulter Electronics ltd., Luton, England) and identified microscopically in a smear. Mononuclear cells were isolated according to Elin and Johnson [5]. The heparinised blood was diluted with equal volumes of

buffered saline and glucose solution (BSG; containing 8.1 g NaCl, 1.53 g $NaHPO_4.2H_2O$, 0.194 g $NaH_2PO_4.H_2O$ and 2 g glucose per litre with an osmolality of 290 mosm/kg and a pH 7.4 adjusted with NaOH). The cord blood was diluted with 20 mL of BSG because of the high hemoglobin levels and viscosity. Then, the mixture was carefully layered onto Ficoll-Paque (density 1.077 kg/L) and centrifuged at 400 x g for 35 min. The interfase was collected, washed in BSG, and centrifuged at 400 x g for 10 min. To the pellet we added 4.5 mL BSG and counted the leukocytes. A paraformaldehyde fixation in a cytospin preparation was made and the

Table 1. Reference values (highest, lowest and (median)) for the various age groups.

Group	I (n = 8)	II (n = 8)	III (n = 14)	IV (n = 57)
sc-Mg				
mmol/L	0.64-0.77 (0.75)	0.70-1.15 (0.87)	0.78-1.04 (0.87)	0.65-0.99 (0.89)
mo-Mg				
fmol/cell	2.96-6.50 (4.04)	2.96-7.00 (6.10)	2.42-5.79 (4.08)	1.38-7.75 (3.63)
µmol/g prot	42.5-49.6 (46.3)	16.3-52.1 (35.6)	33.3-50.4 (40.8)	22.1-76.3 (37.1)
mmol/g DNA	0.43-1.05 (0.54)	0.35-1.08 (0.49)	0.37-0.85 (0.51)	0.12-0.97 (0.47)
mo-K				
fmol/cell	18.3-75.6 (35.4)	19.3-74.2 (51.4)	25.8-63.6 (34.3)	20.2-68.4 (32.4)
mmol/g prot	0.29-0.51 (0.40)	0.25-0.56 (0.32)	0.21-0.68 (0.36)	0.21-0.53 (0.33)
mmol/g DNA	2.92-12.13 (4.71)	2.28-11.23 (4.83)	2.82-10.31 (4.31)	2.67-8.56 (4.13)
mo-Protein				
pg/cell	63.6-151.6 (86.6)	56.9-297.0 (158.6)	49.6-158.3 (106.7)	54.1-205.0 (98.3)
mo-DNA				
pg/cell	6.0-9.0 (7.9)	6.5-15.4 (8.4)	5.3-10.4 (8.1)	5.2-11.4 (7.8)
er-Mg				
µmol/g RBC	5.04-5.54 (5.28)	4.67-5.77 (5.45)	6.08-8.77 (7.29)	5.23-8.08 (6.42)
er-K				
mmol/g RBC	0.23-0.27 (0.24)	0.20-0.27 (0.25)	0.25-0.29 (0.26)	0.21-0.30 (0.21)

viability of cells was checked by staining with tryptan blue [6]. Exactly 4 mL of the cellsuspension was centrifuged at 600 x g for 10 min. To the pellet we added 700 μL of de-ionised water and sonicated the sample three times, 15 s each at 45 s interfal. The tubes were cooled in ice. The magnesium content of the lysate was determined with atomic absorption spectrophotometry using lanthanoxide [5]. Protein was measured according to Lowry [7]. DNA was estimated with use of DAPI (4,6 diamidino-2-phenylindole.2HCl) [8]. From the counted total number of mononuclear cells in the known volume of venous blood and in the cell suspension, we calculated the percentage of harvested mononuclear cells. Magnesium was expressed as fmol/cell, μmol/g of protein and mmol/g of DNA. The potassium content of the lysate, measured by flame photometry was expressed as fmol/cell, mmol/g of protein and mmol/g of DNA.

After centrifugation, erythrocytes were collected and washed four times in CsCl (155 mmol/L, osmolality 290 mosmol/kg) [9]. We then lysed 100 μL of packed cells in 400 μL of de-ionised water. We suspended 100 μL from this lysate in an Eppendorf cup, evaporated the liquid and weighted the residue. The lysate was frozen untill time of assay. Magnesium and potassium were expressed as micromoles or millimoles, respectively, per gram of erythrocytes dry weight.

Statistical methods

We used Spearman's rank correlation coefficients to detect possible relationships between different variables. The Kruskal-Wallis test [10] was used to detect possible differences between the age groups for all analytes. Control for normality was performed according to Stephens [11], and we used Wilcoxon's two sample test to detect possible differences between boys and girls. Results were considered to be significant when $p<0.05$.

RESULTS

Microscopic identifying and counting of the harvested cells gave: 1% ± 2(mean + SD) polynuclear cells, 85% ± 6 lymphocytes, and 13% ± 6 monocytes. From the total amount of mononuclear cells in the venous blood, 56 ± 13%(mean + SD) were harvested by this Ficoll-Paque isolation technique. The viability test with tryptan blue showed 95-98% vital cells.

Because significant differences in any parameter were absent (Kruskal-Wallis test: $p>0.05$) in all age-groups above the age of six months, all children older than six months were combined into one group (IV).

Table 1 lists the reference values of the different groups. Because of the limited number of samples in Groups I - III, we list the highest, lowest and median value. Serum magnesium (fig.1) in cordblood (Group I) was significantly lower than in samples from age groups older than one month (Group III and IV), with $p< 0.01$ for both groups, but showed no difference from results of samples collected during the first month post partum (Group II). By the first month, there was a wide spread in the results and a considerable increase in serum magnesium to a median value of

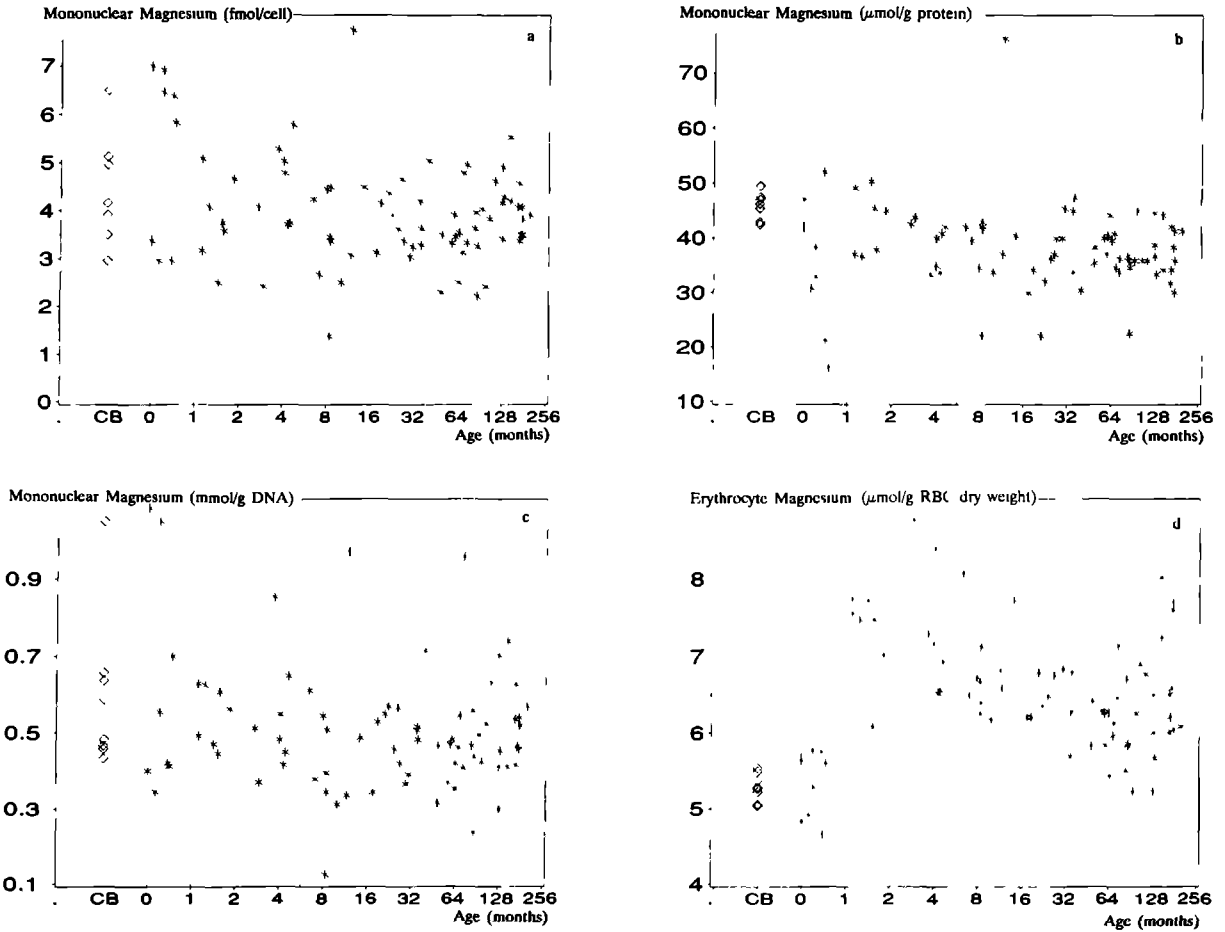

Figure 2. Mononuclear cell magnesium content vs age, expressed as fmol/cell (a), μmol/g of protein (b) and mmol/g of DNA (c); and (d) erythrocyte magnesium content vs age. CB, cord blood.

0.87 vs 0.75 mmol/L in cord blood.
Figure 2 a-c shows concentrations of magnesium in mononuclear cells (mo-Mg) for the whole population versus age. mo-Mg expressed as fmol/cell and mmol/g of DNA was not significantly different between all groups; but when expressed as mmol/g of protein, mo-Mg of Group I was significantly higher than that in Group III and IV ($p=0.015$ respectively $p<0.01$), probably because of a smaller range and lower median cell protein content in Group I. For all samples, mo-Mg in fmol/cell correlated very well with mo-Mg expressed as mmol/g of DNA ($r=0.82$, $P<0.01$), but no significant correlation was found between mo-Mg was expressed as fmol/cell or mmol/g of DNA versus mmol/g of protein. mo-Mg expressed in all three ways had no significant correlation with se-Mg.
Erythrocyte magnesium content (figure 2d) was significantly lower in Groups I and II in comparision with Groups III and IV ($p<0.01$ for all instances). In contrast to the magnesium concentrations in serum, which rose during the first month post partum, the erythrocyte magnesium content increased between one and six months of age. This is reflected by the median erythrocyte magnesium content of 5.28 and 5.45 μmol/g RBC in Groups I and II respectively, increasing to 7.29 μmol/l RBC in Group III.

Table 2. Correlation between magnesium and potassium in mononuclear cells (both expressed per cell, protein and DNA content) and erythrocytes.

		r	p
Mononuclear cells	fmol/cell	0.65	0.0001
	μmol/g protein	0.32	0.0042
	mmol/g DNA	0.73	0.0001
Erythrocytes	μmol(vs mmol/g RBC dry weight	0.28	0.0170

We also studied mononuclear cell potassium (mo-K) in the different age groups but found no changes with age. The relation between mo-Mg and mo-K is presented in figure 3a and table 2. A remarkable lower correlation coefficient between these cations existed when mo-Mg and mo-K were expressed in mmol/g of protein, compared with the expression in fmol/cell and mmol/g of DNA (table 2).
Both mo-Mg and mo-K content showed significant correlations with mononuclear protein content ($r=0.73$, $p=0.01$ and $r=0.50$, $p=0.01$ respectively), pointing to the influence of cell composition and size on magnesium and potassium content of mononuclear cells. On the other hand, we detected no significant correlation of mo-Mg and mo-K content with the DNA content of the cells ($r= 0.08$, $p=0.31$ and $r=0.01$, $p=0.56$ respectively).
er-K showed no significant differences in all groups.
er-Mg and er-K showed a positive but low correlation coefficient ($r=0.28$ $p<0.05$)(fig. 3b and table 2).
A significant correlation between mononuclear cells and erythrocytes for both

magnesium and potassium was absent.

DISCUSSION

Although reference values for mononuclear magnesium and potassium have been collected in adults, no data are known regarding childhood. Only Paunier et al. [3] investigated the fetal-maternal relationship of intra- and extracellular magnesium and potassium concentrations. The cord blood data in this study were comparable to those presented by Paunier et al. [3] but the range that we found was smaller. From published data for adults [2,12,13,14,15,16], the mo-Mg content of children appears to be in the same range as reported for adults (2.00 - 4.83 fmol/cell and 36.6 - 66.8 μmol/g of protein) [12]. Our results for mo-Mg expressed as mmol/g of DNA are a little higher than reported for adults (0.25- 0.57) [16], whereas mononuclear DNA

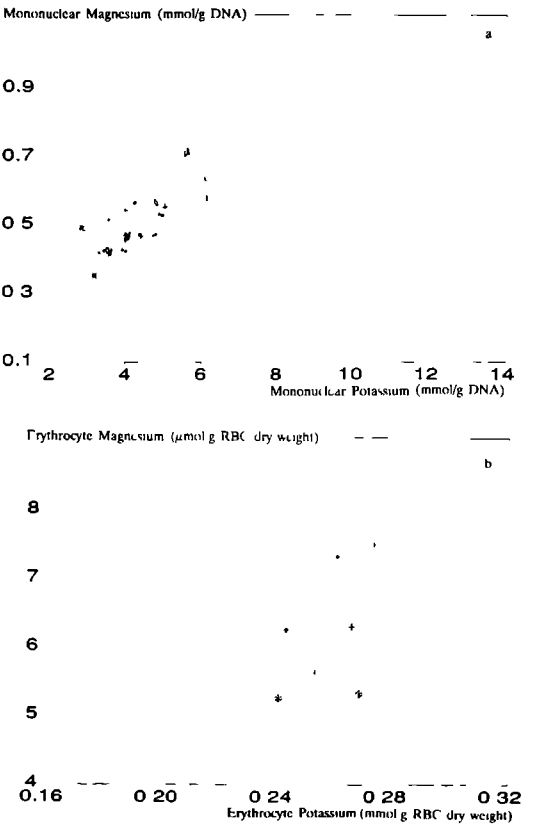

Figure 3. Correlation of magnesium and potassium content in (a) mononuclear cells ($r=0.82$ $p<0.01$) and (b) erythrocytes ($r=0.28$, $p<0.05$) ◇, cord blood.

contents were in the same range. As Elin et al. [12,16] have pointed out, expressing mononuclear magnesium in terms of protein content leads to significant problems. We agree with Elin and Hosseini [16] that, because of the considerable differences in cellular protein content and the wide ranges in cell size, which possibly lead to fortuitously noticeable differences between age groups, expression in fmol/cell or mmol/g of DNA is preferable. The conclusion that age does not influence mononuclear magnesium content leaves the expression in terms of protein, out of consideration. Like most other authors [4,5,12-15] except Ryzen et al.[17], we detected no correlation for magnesium between serum and mononuclear blood cells. Only a few data are available about erythrocyte magnesium in childhood [3,18,19]. Besides the wide range shown in all papers, every author uses an other unit to express the erythrocyte magnesium amount. Table 3 shows a survey of these data, with a conversion into the unit(-type) used by us, based on the assumption of a protein content of 371 g/L and dry weight 402 g/L of erythrocytes, for adult erythrocytes [20,21]. In cord blood and during the first weeks of life, the mean corpuscular volume of erythrocytes is higher than in adults [25], which effects total protein content and dry weight/cell. We prefer, however, to relate the erythrocyte magnesium content to grams of erythrocytes dry weight because two major sources of error associated with the usual technique by which the magnesium content is related to the the original volume of packed cells [9]. First, variations in the quantity of trapped extracellular fluid should be taken into account; secondly, because of the extreme viscosity of packed cells, accurate quantitative pipetting is almost impossible.

The physiological meaning of this way of expression (μmol/g of erythrocytes, dry weight), however, is doubtful because the erythrocytes in infants younger than one month of age have a higher mean corpuscular volume than those in older infants and children. Therefore, the number of erythrocytes per gram erythrocytes dry weight is lower in this age group, which contributes to the lower magnesium content in Groups I and II.

Table 3. Erythrocyte magnesium content reported in the literature and this study.

	units	mean ± 2 sd	µmol/g RBC (dry weight)
adults			
[13]	nmol/mg prot.	6.37-9.57	2.38-3.54
[18]	mmol/L RBC	1.43-2.99	3.54-7.56
[23]	mmol/L RBC	1.69-2.99	4.21-7.56
[24]	mmol/L RBC	1.71-2.95	4.25-7.33
[25]	µg/g RBC dry weight	125-191	5.21-7.96
children > 6 months			
[18]	mmol/L RBC	1.53-3.69	3.79-9.17
This study (range)			5.23-8.08

The relationship of intracellular magnesium and potassium in humans was investigated in three studies [3,4,13], one of which [3] studied cord blood. Mononuclear magnesium and potassium were highly correlated in adult and cord blood mononuclear cells in two of these studies [3,13] but not in the other [4]. Our study confirms that this correlation is real and exists not only in mononuclear cells of adults and cord blood but also in mononuclear cells of children.
Determination of the reference range for different age groups of children will allow meaningfull studies in disorders of potassium and magnesium metabolism in childhood.

ACKNOWLEDGEMENTS

This study was supported by the Dutch Kidney Foundation.

REFERENCES

1. Baron DN, Ahmed SA. Intracellular concentrations of water and the principal electrolytes determined by analysis of isolated human leucocytes. Clin Sci 1969,37.205-19.
2. Elin R. Status of the determination of magnesium in mononuclear blood cells in humans. Magnesium 1988;7 300-5.
3. Paunier L, Giardin E, Brioschi PA, Béguin F Maternal-fetal relationship of extra- and intracellular magnesium and potassium concentration. In: Altura BM, Durlach J, Seelig M eds , Magnesium in cellular processes and medicine, Basel Karger, 1987,151-5
4. Abraham S A, Rosenman U.E D, Meshulam Z, Brisk R. Lymphocyte and erythrocyte concentration of potassium magnesium and calcium in normal controls. Magnesium 1985,4:102-5
5. Elin RJ, Johnson E. A method for the determination of the magnesium content of mononuclear cells Magnesium 1982;1:115-21.
6. Phillips HJ. Dye exclusion test for the viability, In Kruse PF, Patterson MK eds Tissue culture: Methods and Applications, New York NY. Academic Press, 1983 407-8.
7. Lowry O, Rosenbraugh N, Randall R. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
8. Kapuscinski J, Skozylas B. Simple and rapid fluorimetric method for DNA microassay Anal Biochem 1977,83 252-7.
9. Weisberg PL, West MJ, Woods KL. An improved method for measuring intracellular electrolytes and effect of cold storage. Clin Chim Acta 1983,129 85-9.
10. Kruskal W. A nonparametric test for the several sample problem Ann Math Statist 1952,23·525-40.
11. Stephens MA. EDF statistics for goodness of fit and some comparisons. J Am Statist Ass 1974;69:730-7.
12. Hosseini J M, Johnson E, Elin R. Comparison of two separation techniques for the determination of blood mononuclear cell magnesium content. J Am Coll Nutr 1983,4.361-8.
13. Giardin E, Paunier L. Relationship between magnesium, potassium and sodium concentrations in lymphocytes and erythrocytes from normal subjects. Magnesium 1985,4 188-92.
14. Ryan M P, Ryan M F, Thornton L, Counihan T.B. The use of lymphocytes to monitor cellular magnesium and potassium. Mag-Bull 1981,2:113-6.
15. Reinhart R A, Marx J.J, Haas R G, Desbiens N,A. Intracellular magnesium of mononuclear cells from venous blood of clinically healthy subjects. Clin Chim Acta 1987,167 187-95

16. Elin R.J, Hosseini J.M. Magnesium content of mononuclear blood cells. Clin Chem 1985;31:377-80.
17. Ryzen E, Elkayam U, Rude R.K. Low blood mononuclear cell magnesium in intensive cardiac care unit patients. Am Heart J 1986;111:475-80.
18. Feenders O, Dominck H Chr, Bachmann KD. Die Magnesium-konzentration der Erythrozyten und des Plasmas in kindersalter. Dtsch med Wschr 1977;102:1065-7.
19. Leeuw I de, Vanroelen W, Vertommen J. Cordblood Mg in normal pregnancy. Abstract. Magnesium-Bulletin 1981;1:41.
20. Beutler E. Composition of the erythrocyte. In: Williams W.J, Beutler E, Erstev A, Lichtman M.A, eds. Hematology, 3rd edition. New York: McGraw-Hill; 1983, 280-8.
21. Bernstein R.E. Alterations in metabolic energetics and cation transport during aging of red cells. J Clin Invest 1959;38:1572-86.
22. Guest G.M, Brown E.W. Erythrocytes and hemoglobin of blood in infancy and childhood III. Am J Dis Child 1957;93:486-9.
23. Archer W.H, Emerson R.L, Reusch C.S. Intra and extracellular fluid magnesium by atomic absorption spectrophotometry. Clin Biochem 1972;5:159-61.
24. Nelson D, Henningsen N.C. Erythrocyte contents of electrolytes (Na, K, Mg, Zn) in healthy male controls and offspring to established hypertensive patients: a follow-up study. Scand J Clin Lab Invest 1983;43:317-22.
25. Geven W.B, Thomas C.M.G, Vogels-Mentink G.M, Willems J.L, Monnens L.A.H. Renal Prostaglandin excretion in patients with isolated hypomagnesemia. Mag-Bull 1989;11:64-7.

Chapter 6

EXPERIMENTAL MAGNESIUM DEPLETION IN THE DOG

Influence on the magnesium content of mononuclear cells, erythrocytes, muscle and bone

Published in Magnesium Bulletin 1988; 10: 45-50.

EXPERIMENTAL MAGNESIUM DEPLETION IN THE DOG

Influence on the magnesium content of mononuclear cells, erythrocytes, muscle and bone

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SUMMARY

In experimental deficient dogs data were obtained of serum magnesium and mononuclear, erythrocyte, bone and muscle magnesium amounts, before and during deficiency state and in half of the dogs after restoration of the serum magnesium level. In each dog 6, respectively 7 measurements were performed. The results showed a significant decrease of serum, erythrocyte and bone magnesium during and at the end of the magnesium deficiency. Highly positive correlation coefficients for serum and erythrocyte magnesium were found and positive correlation was shown for erythrocyte and bone magnesium amounts. No decrease of the magnesium level in muscle and mononuclear cells was shown during and at the end of magnesium deficiency, but significant rise of muscular magnesium was found after normalisation of serum magnesium. In magnesium deficiency state erythrocyte magnesium levels appeared to be a better reflection of the body magnesium stores than mononuclear cells.

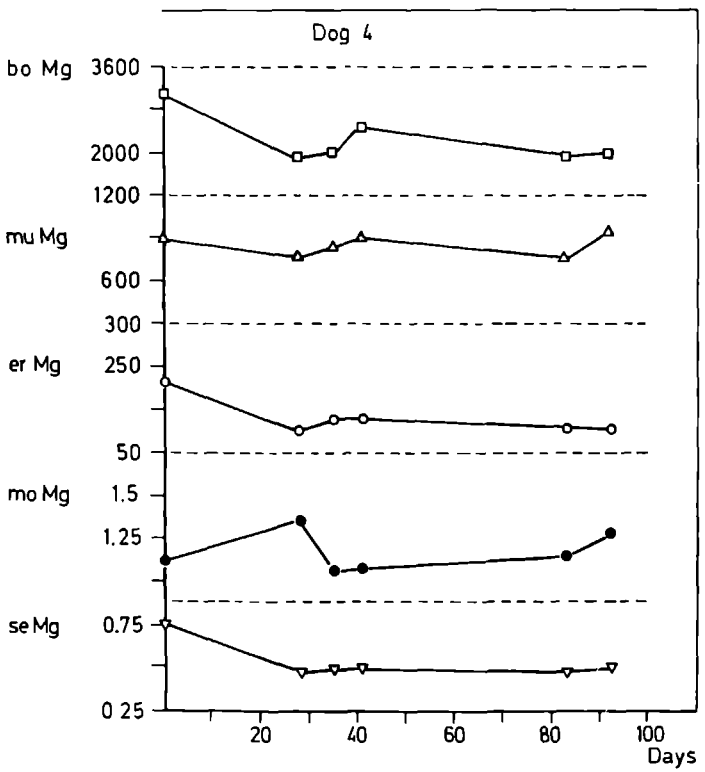

Figure 1. The total experimental period of dog 4.
Magnesium in se-Mg is expressed in mmol/l, mo-Mg in µg/mg protein, er-Mg in µg/gr RBC dry weight, mu-Mg in µg/gr dry weight and bo-Mg in µg/gr dry weight.

INTRODUCTION

Magnesium is distributed in three major compartments of the body: 65% in the skeleton, 34% in the intracellular space and only 1% in the extra-cellular fluid. In their seminal study in man about body magnesium stores, Alfrey et al. [1] concluded that bone and extracellular fluid magnesium are the major magnesium pools decreased during magnesium depletion. There was a highly significant correlation between the levels of serum and bone magnesium (se-Mg and bo-Mg respectively). Muscle magnesium content did not reflect total body magnesium [1].
Baron and Ahmed [2] and Ryan et al. [15] advocated to use peripheral blood lymphocytes for the estimation of intracellular electrolytes. During experimental Mg deficiency in rats the magnitude of the Mg loss from the lymphocytes was similar to that of cardiac and skeletal muscle tissue [16].
The aim of our study was to reevaluate the significance of se-Mg and magnesium content of erythrocytes (er-Mg), muscle (mu-Mg),bone (bo-Mg) tissue and also of mononuclear cells (mo-Mg), for the monitoring of total body magnesium in an experimental study of magnesium depletion in the dog. The size of the animal allowed us repeated sampling in the same animal. In agreement with most data bo-Mg is closely related to the extracellular magnesium content while mu-Mg and mo-Mg remain relatively stable. The magnesium content of erytrocytes showed a positive correlation with both se-Mg and bo-Mg. Futher studies of the subcellular distribution of magnesium in the erytrocyte should provide the required knowledge for application in clinical studies.

MATERIAL AND METHODS

Ten young adult beagles, housed in individual cages, were fed with a magnesium deficient diet and de-ionised water. Table 1 and 2 show the composition of the diet

Table 1. Composition of the diet without supplemented MgO, containing 170 ppm Magnesium.

Ingredient:	%
Casein	32.0
Corn starch	28.0
Dextrose	26.0
Lard	4.0
Soybean oil	1.0
Cellulose	3.0
Dicalcium phosphate	1.67
Calcium carbonate	1.67
NaCl	0.43
dl-Methionin	0.20
KH_2PO_4	1.06
Vitamin and trace element mixture	0.77
Choline chloride	0.20

that contained 170 ppm magnesium. During the first 4 weeks, the magnesium intake was gradually lowered weekly from 4.5 via 4.0 and 3.5 to 3.25 mg magnesium/kg body weight per day. In 5 dogs the magnesium intake was increased by supplementation with MgO (12mg Mg/kg.day) after 112 days. Each week blood was drawn for measurement of magnesium, sodium, potassium, calcium, phosphate, ureum, creatinine, hemoglobine and leucocytes. When the se-Mg levels had dropped to about 0.5 mmol/l (normal 0.70-1.1) [14] samples were taken and data were collected 3 times with intervals of one week. Thereafter magnesium deficient diet was maintained and one month later blooddrawing and biopsies were repeated twice with one week interval.

In 5 dogs in whom the intake of magnesium had been restored, analyses of se-Mg, mo-Mg, er-Mg and a seventh bone and muscle biopsy were done, one week after normalisation of se-Mg levels.

Blood was drawn from the dog in a conscious state while biopsies were taken during narcosis with droperidol/fentanyl followed by pentobarbital. Bonebiopsy was a punchbiopsy with a diameter of 0.5 cm from the iliac crest, carefully selecting a new biopsy site at each puncture. Muscle biopsy was taken from the musculus gracilis.

Table 2. Vitamins and trace elements added to one kilogram of the magnesium deficient diet.

Vitamin A	15000	IU
Vitamin D_3	1500	IU
Vitamin K_3 (menadione)	2.5	mg
Vitamin E	92	mg
Thiamine hydrochloride	20	mg
Riboflavin	20	mg
Pyridoxine HCl	15	mg
Niacin	40	mg
d-Ca-Pantothenate	40	mg
Vitamin B_{12}	40	µg
Biotin	200	µg
Folic acid	5	mg
Inositol	200	mg
Iron subcarbonate (57% Fe)	180	mg
Copper carbonate (55% Cu)	30	mg
Manganous oxide (62% Mn)	100	mg
Zinc oxide (78% Zn)	71	mg
Na Selenite (45% Se)	400	µg
$Ca(IO3)_2$	1.0	mg

LABORATORY METHODS

Mg was measured by atomic absorption spectrophotometry (Perkin Elmer). Other measurements were done by routine laboratory methods. Isolation of mononuclear cells was performed according to Elin and Johnson [7]. Ten ml heparinised blood was diluted with equal amounts of a buffered saline and glucose solution (BSG), carefully

layered on Ficoll-Paque (brought on a densitiy of 1070) and centrifuged at 400 x g for 35 minutes. The interface was collected, washed in BSG and centrifuged at 400 x g for 10 minutes. To the pellet 4.5 ml BSG was added and leucocytes were counted on a Coulter Counter (Coulter Counter Electronics). A cytospin preparation was made and the viability of cells was chequed by a supravital staining with tryptan blue. Exactly 4 ml of the cell suspence was centrifuged at 600 x g for 10 minutes. To the pellet 700 µl de-ionised water was added and sonicated for 3 x 15 seconds with 45 seconds intervals.

The magnesium content of the lysate was determined with atomic absorption spectrophotometry. Protein was measured according to Lowry. DNA was estimated with DAPI (4,6 diamidino-2-phenylindole.2HCl) [11]. Magnesium content was expressed as µg/mg protein and as µg/ mg DNA.

Erythrocytes: after centrifugation erythrocytes were collected and washed 4 times in CsCl(155 mmol/l, osmolarity: 290 mosmol/kg) [22]. Then 100 µl packed cells were lysed in 400 µl de-ionised water. From this lysate 100 µl was suspended in separate cups, dried and weighted. The lysate was frozen untill time of assay. Magnesium content was expressed as µg/g. RBC (dry weight).

After the biopsy was taken, the bone was cleaned from blood rests in H_2O_2 3% for 1 minute, the samples rinsed with de-ionized water, frozen at -70° C, freeze-dried in vacuo and pulverised. After hydrolysis with concentrated HNO_3-$HClO_4$, the obtained solution was supplemented with de-ionized water and the magnesium concentration measured [3]. Magnesium was expressed as µg/g.dry weight.

Muscle was handled exactly as bone except the H_2O_2 procedure. The magnesium content was also expressed as µg/g.dry weight.

Table 3. Mean and standard deviation of the measurements in all dogs before and at the end of the magnesium deficiency period.

n=10	before		end		p
	mean	(sd)	mean	(sd)	
se-Mg	0.77	(0.06)	0.45	(0.11)	<0.01
mo-Mg µg/mg prot.	1.15	(0.07)	1.09	(0.17)	ns
mo-Mg µg/mg DNA	41	(16)	32	(15)	ns
er-Mg	212	(16)	106	(19)	<0.01
mu-Mg	871	(99)	727	(162)	ns
bo-Mg	2635	(539)	1896	(389)	<0.01

Magnesium in se-Mg is expressed in mmol/l, mo-Mg:µg/mg protein and in µg/mg DNA, er-Mg in µg/gr RBC dry weight, mu-Mg in µg/gr dry weight and bo-Mg in µg/gr dry weight.

Statistical methods

We used Wilcoxon's signed rank test to detect differences between different determined values and Spearman's rank correlation coefficients to detect possible

relationships between different variables. Results were considered to be significant when $p < 0.05$.

RESULTS

Between the dogs there are differences in the rate of lowering of the se-Mg concentration. The second measurement of mo-Mg, er-Mg, mu-Mg and bo-Mg was undertaken in 5 dogs between 35-40 days and in the others between 50-63 days after the start of magnesium deficient diet. Sodium, potassium, calcium, ureum and creatinine levels remained within the normal range in every dog during the entire experiment. Phosphate levels were slightly lower at the end of the experiment in most dogs but remained close to the lower range of normal values. Figure 1 shows the results in one dog.

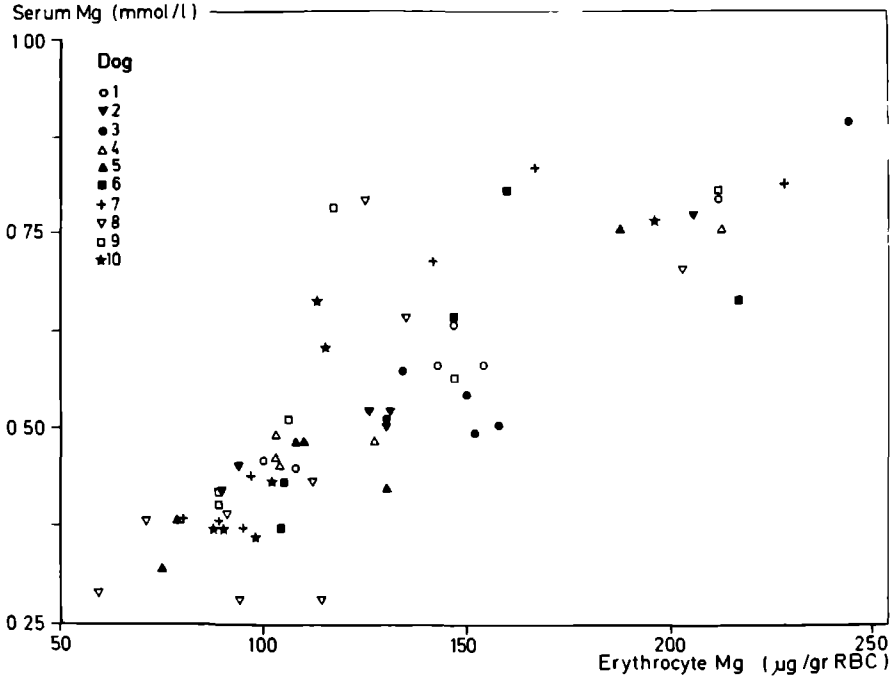

Figure 2. se-Mg (mmol/l) and er-Mg (µg/gr RBC) show good correlation.

No significant difference for bone, muscle, erythrocyte, mononuclear cell content and serum magnesium between the measurements after 35-40 days and 1 month later was

observed. Table 3 presents the data of analyses performed before and at the end of magnesium deficiency. Data before and during magnesium deficiency presented as the mean of the measurements 2 - 6 are shown in table 4. The results after normalisation of se-Mg are a cumulation of 5 determinations. In table 5 data are shown one week after supplementation of magnesium. In table 6 median individual correlation coefficients and their range for these compared data are listed.

Mononuclear cells

There was no significant change in mo-Mg during the periode of Mg deficiency while mo-Mg (µg/mg.protein) showed significant rise after supplementation. Individual correlation coefficients for mo-Mg showed a very wide range and the medians of the individual correlation coefficients are mostly weak. In none of the dogs a significant positive correlation between mo-Mg/mu-Mg and mo-Mg/bo-Mg could be demonstrated.

Erythrocytes

In figure 2 se-Mg is plotted against er-Mg. A significant lowering during deficiency and a significant rise after normalisation of se-Mg was demonstrated (table 3,4 and 5).

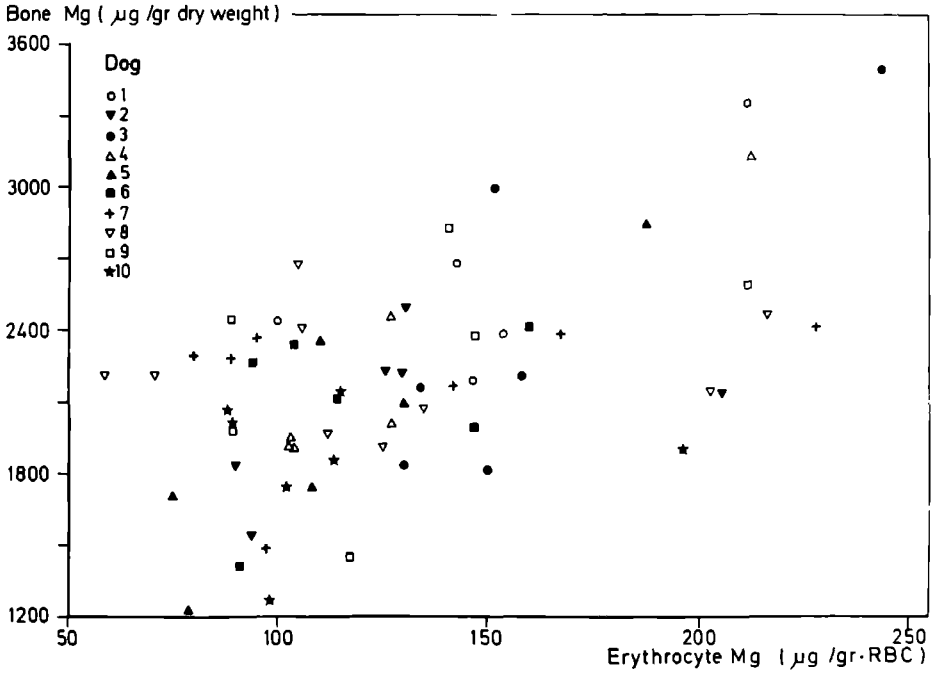

Figure 3. Bo-Mg (µg/gr dry weight) and er-Mg (µg/gr RBC) have a low correlation.

A high correlation was shown for er-Mg and se-Mg (table 6 and figure 2). Six dogs had significant individual correlation coefficients (0.81-1.0; $p<0.05$) and in the others it was also positive. Low positive median individual correlation coefficient was present for er-Mg and bo-Mg (fig.3, table 6).

Muscular magnesium

The muscular magnesium content of Mg did not drop significantly during the deficiency state but raised significantly after normalisation of se-Mg (table 3,4 and 5). Individual correlation coefficients between mu-Mg/er-Mg and mu-Mg/bo-Mg were not significant in any of the dogs and the median showed very wide ranges (table 6).

Bone magnesium

Among the dogs, bone magnesium contents showed a wide range at the beginning of the experiment (3479 - 1895 μg/gr. dry weight) when se-Mg was normal. In the dogs

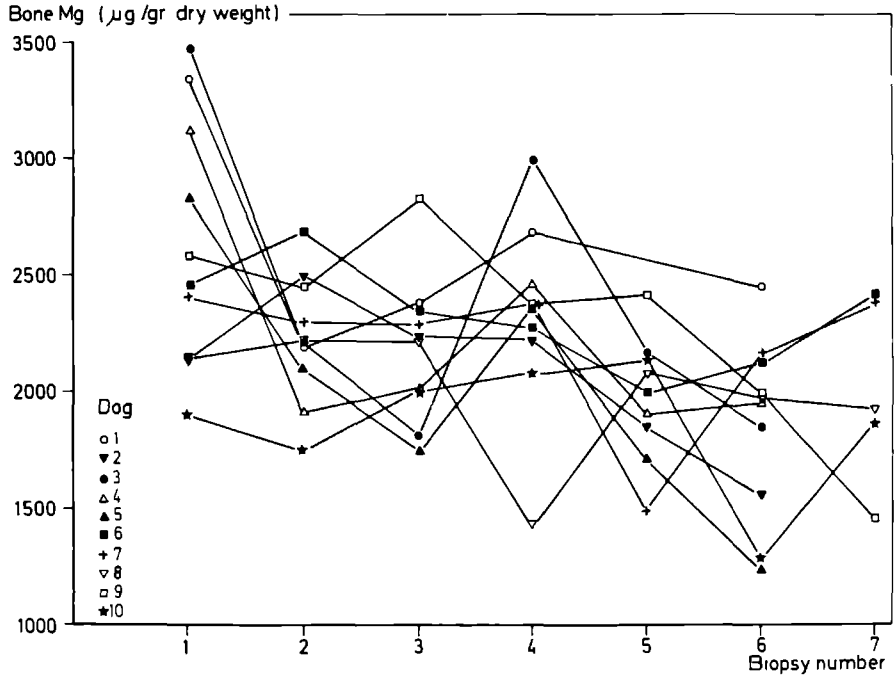

Figure 4. Bone magnesium content of all dogs before (biopsy 1) and during the magnesium deficiency (biopsy 2-6).

Table 4. Mean and standard deviation of the measurements in all dogs before and during the magnesium deficiency expressed as the mean of the values found in the biopsies 2-6.

n=10	before		mean 2-6		p
	mean	(sd)	mean	(sd)	
se-Mg	0.77	(0.06)	0.46	(0.05)	<0.01
mo-Mg µg/mg prot.	1.15	(0.07)	1.09	(0.07)	<0.05
mo-Mg µg/mg DNA	41	(16)	41	(22)	ns
er-Mg	212	(16)	112	(22)	<0.01
mu-Mg	871	(99)	760	(82)	ns
bo-Mg	2635	(539)	2114	(209)	<0.01

Magnesium in se-Mg is expressed in mmol/l, mo-Mg:µg/mg protein and in µg/mg DNA, er-Mg in µg/gr RBC dry weight, mu-Mg in µg/gr dry weight and bo-Mg in µg/gr dry weight.

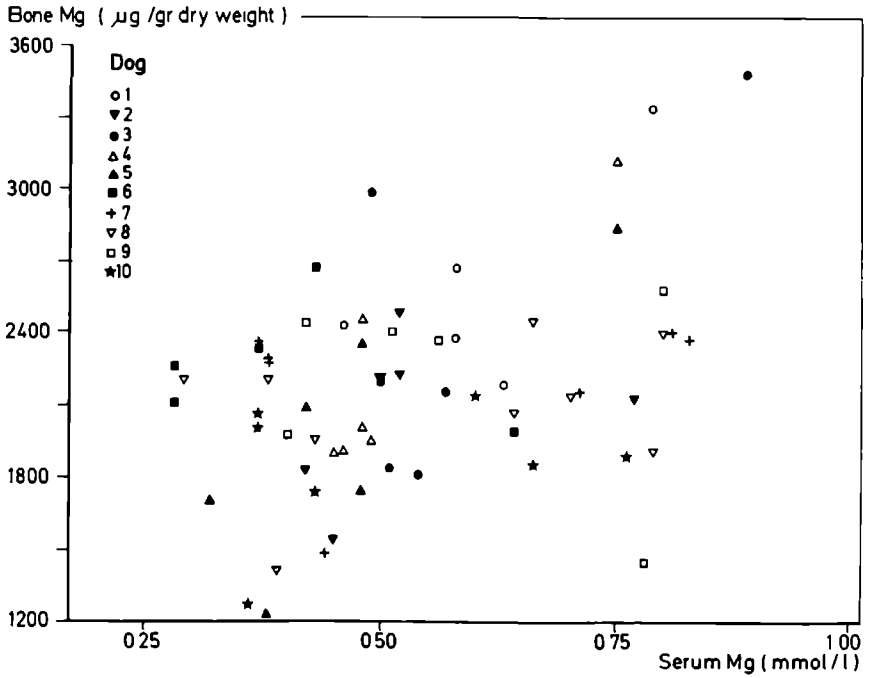

Figure 5. Bo-Mg (µg/gr dry weight) and se-Mg (mmol/l) have a low correlation.

with high bo-Mg lowering took place in the first 30-50 days of the deficiency period, but dogs with low bo-Mg showed only a slight decrease of bone magnesium content during the entire deficiency period (fig.4). In figure 5 bo-Mg is plotted against er-Mg.

Median correlations of se-Mg and er-Mg or bo-Mg are positive but the range of individual correlation coefficients is wide (fig. 3, 5; table 6).

DISCUSSION

Though all dogs developed depressed se-Mg levels and one or more signs of magnesium deficiency (ragged hair, hypotonia of behind legs, anorexia or slight weight loss), we were unable to find a drop in muscular or in lymphocyte magnesium content but a significant lower bo-Mg. Even in severe and prolonged magnesium deficiency Cronin et al. [5] established only modest lowering of muscle magnesium content in the dog, with means that muscle cells and lymphocytes must have protective mechanisms against intracellular magnesium deficiency. In patients and healthy men contradictory data have been reported about the relationship between the amount of muscular and mononuclear magnesium [6,17,19]. Recently Wallach [20] reviewed magnesium exchangeability in magnesium deficiency and reported about the very low exchangeable bone magnesium in vivo of 1-2% and a decrease of bone magnesium content of 17% in magnesium deprived dogs. The wide range of the bo-Mg levels before the start of the Mg deficiency indicate that individual measurements of bone magnesium content have limited applicability for the detection of intracellular magnesium deficiency. In contrast with Cohen and Kitzes [4] who found a very good correlation between se-Mg and bo-Mg in Mg deficient patients, we were unable to confirm this in Mg deficient dogs.
Alfrey et al. [1] already demonstrated that erytrocyte magnesium levels in general correlated well with the serum magnesium level. They stressed the compounding influence of the erythrocyte age on the magnesium content. Younger cells have a higher magnesium content than older cells [16]. In magnesium deficiency the erytrocyte survival is reduced [8,10]. This should cause a raise in the average red cell magnesium content. Further studies on the distribution of Mg inside the erythrocytes, using NMR techniques, should therefore be initiated. The Mg content of the erythrocyte could be of value in monitoring of body magnesium content.

Table 5. Mean and standard deviation of the measurements in 5 dogs at the end of the magnesium deficiency and when the se-Mg had become normal after supplementaion with MgO.

n=5	end		restored se-Mg		p
	mean	(sd)	mean	(sd)	
se-Mg	0.44	(0.16)	0.77	(0.07)	<0.05
mo-Mg µg/mg prot.	1.04	(0.19)	1.26	(0.12)	<0.05
mo-Mg µg/mg DNA	35	(20)	64	(33)	ns
er-Mg	111	(20)	136	(25)	<0.05
mu-Mg	591	(94)	709	(84)	<0.05
bo-Mg	1896	(358)	1996	(398)	ns

Magnesium in se-Mg is expressed in mmol/l, mo-Mg: µg/mg protein and in µg/mg DNA, er-Mg in µg/gr RBC dry weight, mu-Mg in µg/gr dry weight and bo-Mg in µg/gr dry weight.

Athough it is known that er-Mg is lowered in Mg deficiency [18], there is lack of sufficient information concerning the erthrocyte magnesium content changes during deficiency. In these experiments er-Mg was already lowered when the second biopsy was done (figure 1) in all dogs. In table 3 is shown that the er-Mg levels at the end of the deficiency period and after normalisation of the se-Mg (table 5), is lower than at the beginning of the experiment.
The er-Mg content seems to be no simple reflection of se-Mg. Leinert et al. [12] showed se-Mg changes while er-Mg remained unchanged under acute alcohol load in men and despite elevated se-Mg amounts, normal er-Mg was found in haemodialysis patients [21]. Günther and Vormann [9] describe the impossibility of erythrocytes to take up magnesium in vitro when chicken-erythrocytes had been depleted by divalent cation ionophore A 23187, or in rat-erythrocytes depleted mildly with 2-deoxyglucose or by feeding a magnesium deficient diet. However the timescale between their and our experiment is quite different. Our data (fig. 3) suggest a positive correlation between er-Mg and bo-Mg with was significant in one dog only and nearly significant in 2 others. It is possible that the wide range in bo-Mg at the beginning of the experiment had an influence on this correlation.

Table 6 . Median, minimal and maximal spearman correlation coefficients for compared data in all dogs (biopsies 1-6).

Compared data	r median	r min	r max
se-Mg / mo-Mg µg/mg prot.	0.31	-0.34	0.87
„ / mo-Mg µg/mg DNA	0.50	-0.36	0.70
„ / mu-Mg	0.21	-0.46	0.93
„ / bo-Mg	0.31	-0.42	0.84
mo-Mg µg/mg prot/ mu-Mg	0.03	-0.43	0.25
„ / bo-Mg	-0.03	-0.75	0.54
mo-Mg µg/mg DNA / mu-Mg	-0.31	-0.88	0.26
„ / bo-Mg	0.04	-0.61	0.60
er-Mg / se-Mg	0.81	0.09	1.00
„ / mu-Mg	0.13	-0.37	0.49
„ / bo-Mg	0.30	-0.43	0.88
„ / mo-Mg µg/mg prot.	0.40	-0.79	0.89
„ / mo-Mg µg/mg DNA	0.22	-0.29	0.71
mu-Mg / bo-Mg	0.17	-0.14	0.77

ACKNOWLEDGEMENTS

Our thanks to the members of the Central Animal Laboratory (head: Prof. Dr. W.J.I. v.d. Gulden) for their effective support. This study was supported by the Dutch Kidney Foundation.

REFERENCES

1. Alfrey, A.C., N.L. Miller, D. Butkus: Evaluation of body magnesium stores J. Lab Clin. Med. 84 (1974) 153
2. Baron, D.N. and S.A. Ahmed: Intracellular concentration of water and of principal electrolytes determined by analysis of isolated human leucocytes Clin. Sci. 37 (1969) 205
3. Bayer, W., K. Schmidt: Die Bestimmung von Magnesium in Gewebeproben mittels AAS Mg Bull. 5 (1983) 58
4. Cohen, L., R. Kitzes: Relationship of bone and plasma magnesium in magnesium-deficient cirrhosis patients. Is. J. Med Sci. 18 (1982) 679
5. Cronin, R.E., E.R. Ferguson, A. Shannon, J.P. Knochel· Skeletal muscle injury after magnesium depletion in the dog Am J. Physiol. 243 (1982) F113
6. Dyckner, T., P O. Wester Skeletal muscle magnesium and potassium determinations. correlations with lymphocytes contents of magnesium and potassium. J. Am. Coll. Nutrit. 4 (1985) 61.
7. Elin, R J., E. Johnson: A method for the determination of the magnesium content of blood mononuclear cells. Magnesium 1 (1982) 115.
8. Elin, R.J., A. Utter, H.K. Tan, L. Corash· Effect of magnesium deficiency on erythrocyte aging in rats Am J Pathol 100 (1980) 765
9. Gunther, T, J. Vorman: Removal and Reuptake of intracellular Magnesium Mg Bull. 7 (1985) 66.
10. Heaton, F.W., S. Tongyai, C. Motta, Y Rassiquier, E. Gueux· Changes in the erythrocyte membrane during magnesium deficiency. Nutrit. Res 7 (1987) 655
11. Kapuscinski, J., B. Skozylas. Simple and rapid fluorimetric method for DNA microassay. Anal. Biochem. 83 (1977) 252
12. Leinert, J., P. Becker, D. Hötzel: Veränderungen der Magnesiumgehalte in Serum, Erythrocyten und Harn nach Alkoholbelastung. Mg. Bull. 1 (1981) 42.
13. Nilsson, P., G. Johansson, B.G. Danielsson: Magnesium studies in hemodialysis patients before and after treatment with low dialysate magnesium. Nephron 37 (1984) 25
14. Root, W S., J.B Allison, W H. Cole, J.H. Holmes, W.W. Wolcott, M.I Gregersen: Disturbances in the chemistry and in the acid-base balance of the blood of dogs in hemorrhagic and traumatic shock Am J Physiol 149 (1947) 52
15. Ryan, M.P, M.F. Ryan, L Thonton, T B Lounihan: The use of lymphocytes to monitor cellular magnesium and potassium Mg Bull. 3 (1981) 113.
16. Ryan, M P., M F. Ryan· Lymphocyte electrolyte alterations during magnesium deficiency in the rat. Ir. J. Med Sci. 148 (1979) 108
17. Ryzen, E., U Elkayam, R K. Rude: The use of lymphocytes to monitor cellular magnesium and potassium. Mg. Bull. 3 (1981) 113
18. Shils, M.E.: Experimental production of magnesium deficiency in man. Ann N Y. Acad Sci. 162 (1969) 847
19. Sjögren, A., C-H Floren, A. Nilsson: Measurements of magnesium in mononuclear cells The Science of the Total Environment 42 (1985) 77
20. Wallach, S.: Magnesium exchangeability and bioavailability in magnesium deficiency In· Altura, B M., Durlach, J., Seelig, M S. (eds), Magnesium in cellular processes and medicine Karger (1987) Basel.
21. Watson W.S., T D B. Lyon, TE. Hilditch: Red cell magnesium as a function of cell age. Metabolism 29 (1980) 397
22. Weissberg, P.L., M J West, K.L. Woods· An improved method for maesuring intracellular electrolytes and the effects of cold storage. Clin. Chem. Acta. 129 (1983) 85

Chapter 7

EXPERIMENTAL MAGNESIUM DEPLETION IN THE DOG

Relation between total and ultrafiltrable Mg in plasma and total and ionized Mg in erythrocytes

Published in: Magnesium-Bulletin 1990; 12: 166-169.

EXPERIMENTAL MAGNESIUM DEPLETION IN THE DOG

Relation between total and ultrafiltrable Mg in plasma and total and ionized Mg in erythrocytes

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SUMMARY

Experimental magnesium depletion was induced in dogs by a magnesium deficient diet. A clear divergence was observed between the lowering of plasma magnesium (nadir reached after one week) and the lowering of erythrocyte magnesium content (nadir reached after 3 weeks). As the magnesium stores became more depleted, the erythrocyte content progressively decreased while the lowered plasma level remained stable. The erythrocyte magnesium content in this study behaves as a good indication of body magnesium stores. The low permeability of the erythrocyte membrane for magnesium explains the gap between the nadir of magnesium in plasma and magnesium content of the erythrocyte. Ionized magnesium in the erythrocyte as measured by the ^{31}P NMR technique was clearly diminished after a period of 4 weeks magnesium deficient diet.

INTRODUCTION

In a previous study dealing with experimental Magnesium depletion in dogs, erythrocyte magnesium levels appeared to be a better reflection of the body magnesium stores than mononuclear cells. Due to the wide ranges, bone magnesium levels appeared to be inferior to erythrocyte magnesium content as an estimation of total body magnesium content [4]. As it is well known that red cells have a low magnesium permeability resulting in slow uptake and loss [2] it is important to know whether there will be a divergence in the rate of lowering of plasma magnesium and erythrocyte magnesium content after the introduction of a magnesium deficient diet.
During this study not only plasma magnesium but also ultrafiltrable magnesium, as an approximate value of ionized magnesium, was measured together with ionized magnesium in red blood cells before the start and after 4 weeks of magnesium depletion.

MATERIALS AND METHODS

Ten, one year old inbread beagles were fed with a magnesium deficient diet as previously [4] during one month. The dogs were individually housed and got de-ionised water at libitum. Before and during the experiment heparinised venous blood was drawn weekly at 9 a.m. after an overnight fast. Plasma magnesium was measured by atomic absorption spectrophotometry (Perkin Elmer 5000, Norwalk CT, USA). Ultrafiltrates were obtained after ultrafiltration with Amicon tubes (Amicon Corporation, Danvers,MA, USA) [1]. Erythrocyte total magnesium content was measured in lysed red blood cells as described previously [4], and expressed in $\mu g/g$ red blood cells dry weight or mmol/l cells. Hemoglobin level, hematocrit, number of erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were measured with a H1-jr (Technicon Instruments Corp. Tarrytown, NY, USA). The ionized erythrocyte magnesium level

Table I. Values of extra- and intracellular magnesium before and after 4 weeks of magnesium deprivation.

n=10		Plasma Mg mmol/l mean (sd)	p	Ultra-filtrable Mg mmol/l mean (sd)	p	Erythrocyte Mg µg/g RBC mean (sd)	mmol/l p	mean (sd)	p
day	0	0.75 (0.07)		0.48 (0.08)		171 (11)		2.89 (0.21)	
	7	0.42 (0.10)	<0.01	0.26 (0.07)	<0.01	143 (19)	<0.01	2.44 (0.35)	<0.01
	14	0.38 (0.09)	ns	0.24 (0.07)	ns	112 (21)	<0.01	1.89 (0.33)	<0.01
	21	0.36 (0.11)	ns	0.22 (0.08)	ns	100 (22)	<0.02	1.71 (0.39)	<0.02
	28	0.34 (0.10)	ns	0.24 (0.08)	ns	95 (25)	ns	1.62 (0.45)	ns

was measured by means of ^{31}P NMR as described by Gupta et al [8]. Briefly, ten milliliters of freshly drawn heparinized venous blood was centrifuged and glucose was added to the plasma to a concentration of 10 mmol/l. The plasma was then mixed with the red blood cells and incubated at room temperature for 10 minutes. The blood was centrifuged and an eryhtrocyte-suspension obtained with a hematocrit of ≈ 90 %. Complete oxygenation was ensured with 95% oxygen and 5% CO_2 in a tonometer (Instrumentation Laboratory, Milano, Italy). The wet and dry weights of exactly 0.1 milliliter erythrocyte suspension, with known hematocrit, were measured with a microbalance and waterfraction, dry weight and density of the red blood cells were calculated. The ATP and 2,3-DPG levels in the red blood cells were determined using Sigma diagnostic kits (Sigma Chemical Co, St Louis, USA).

The sample was transferred into a 10 mm outer diameter glass NMR tube with a 4 mm outer diameter coaxial insert containing D_2O for deuterium lock.

^{31}P NMR spectra were recorded at 81 MHz and 37° C on a Bruker WM 200 spectrometer (Bruker, Karlsruhe, FRG) operating in the Fourier transform mode. Pulses of 55° in combination with gated proton decoupling were applied with a repetion time of 0.78 seconds; the samples were spun at 10 Hz during the entire recording.

The calculations of the concentrations of complexes of ATP and 2,3-DPG with Mg^{2+} and /or hemoglobin were done essentially according to Gupta et al. [8] and expressed in mmol/liter cell water. The free fraction of ATP not complexed to magnesium (Φ) is calculated from the NMR spectrum and the equations listed in Gupta's paper.

$$\Phi = \frac{[ATP]_F}{[ATP]_T}$$

Where ATP_F is ATP free and ATP_T is ATP total.

$$\Phi = \frac{(\delta^{cell}_{\alpha\beta} - \delta^{MgATP}_{\alpha\beta})}{(\delta^{ATP}_{\alpha\beta} - \delta^{MgATP}_{\alpha\beta})}$$

In wich $\delta^{cell}_{\alpha\beta}$ is the measured resonance position of the ß-resonance of ATP with respect to the α-resonance, which has a nearly constant position.

$$[Mg^{2+}]_i = Kd^{MgATP} [(1/\Phi) - 1]$$

Where Kd^{MgATP} is the dissociation constant for the reaction

$$MgATP \rightleftharpoons Mg^{2+} + ATP$$

$Kd^{MgATP} = 3.8 \times 10^{-5}$M at 37°C and pH 7.2 [8].

Statistical methods

Spearman's rank correlation coefficients (r) were used to detect relationships between different variables and Wilcoxon's signed rank test to detect differences between variables determined on different data. Results were considered to be significant when $p < 0.05$.

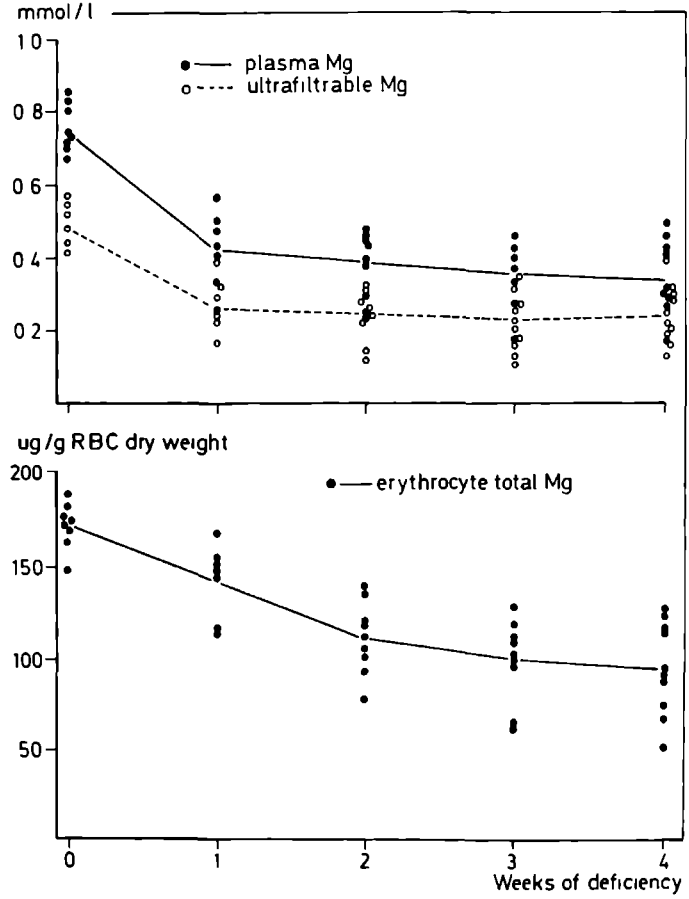

Figure 1. Plasma total, ultrafiltrable and erythrocyte magnesium before and during 4 weeks of magnesium deficient diet show considerable difference in rapidity of decrease between extra-and intracellular magnesium.

RESULTS

Results of plasma-, ultrafiltrable- and total erythrocyte magnesium determinations at day 0, 7, 14, 21 and 28 are shown in table 1. Plasma- and ultrafiltrable magnesium

decreased during the first week of magnesium deficient diet ($p < 0.01$) and remained at a constant level during the rest of the experimental period (fig. 1 upper panel and table 1). Erythrocyte magnesium content, however behaved differently. The lowest level was obtained after three weeks (fig. 1 lower panel). There was a clear delay in time when the lowest eryhtrocyte content was compared with the lowest plasma and ultrafiltrable magnesium (fig. 1).

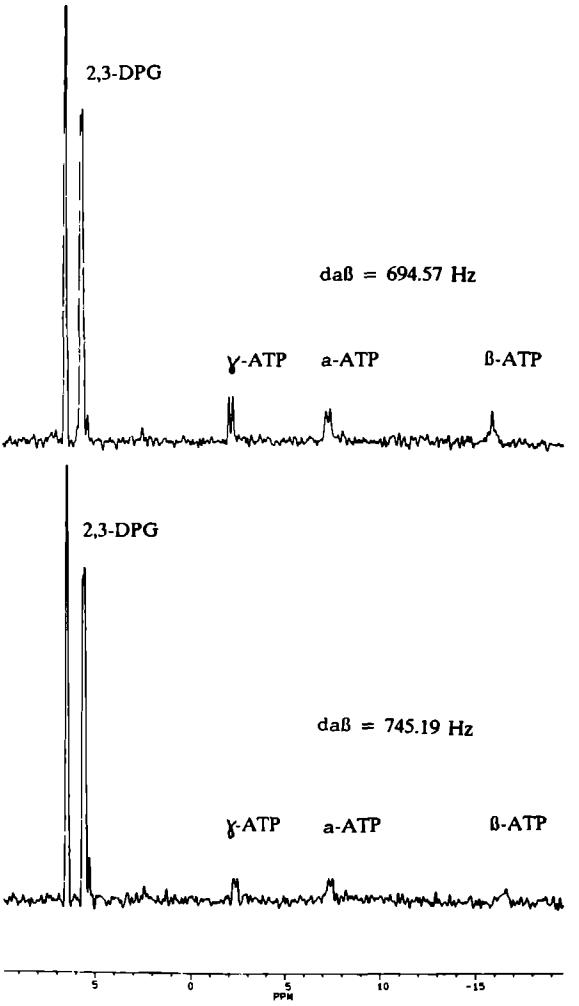

Figure 2. ^{31}P NMR spectra of oxygenated erythrocytes from dog 41 before (upper panel) and after (lower panel) 4 weeks of magnesium deprevation shows a shift in the ß-ATP peak due to decreased intracellular ionized magnesium content.

Figure 2 shows the NMR-spectrum of the erythrocytes before and after 4 weeks of magnesium deficient diet. In contrast to the stable position of the α-ATP peak, there is a clear shift in the ß-ATP peak position after four weeks of magnesium deficient diet, reflecting a difference in intracellular ionized magnesium content.
The ATP content of the erythrocytes was decreased after 4 weeks of deficient diet (Table 2). This presumably is due to the fact that ionized magnesium is an important cofactor for many glycolytic enzymes such as phosphofructokinase [6]. In intact human red cells the intracellular content of ATP decreases as the magnesium content of the cell was reduced [3].
In figure 3 the apparent relation is shown between ionized intracellular magnesium content of the erythrocytes and plasma ultrafiltrable magnesium. Statistical calculations, however, did not show a significant correlation separately on the data before ($r = 0.47$, $p = 0.07$) and after 4 weeks of deficient diet ($r = 0.24$, $p = 0.26$). This is possibly due to the limited numbers of data available.

Table 2. Relevant cellular compounds and characteristics before and after 4 weeks of magnesium deprivation.

n=10	before mean	(sd)	after 4 weeks mean	(sd)	p
Φ	0.132	(0.012)	0.259	(0.071)	<0.01
$[Mg^{2+}]_i$	0.31	(0.03)	0.15	(0.06)	<0.01
ATP	1.12	(0.13)	0.74	(0.15)	<0.01
2,3-DPG	10.36	(0.29)	10.26	(0.85)	ns
fH_2O	0.724	(0.009)	0.718	(0.014)	ns
dry weight	0.407	(0.010)	0.411	(0.015)	ns
density	1.131	(0.014)	1.178	(0.014)	ns
MCV	69	(3)	70	(2)	ns
MCH	1.47	(0.03)	1.51	(0.06)	ns
MCHC	21.2	(0.8)	21.6	(0.9)	ns

$[Mg^{2+}]_i$, ATP, 2,3-DPG in mmol/l cell water, fH_2O, dry weight, density in gram per ml red blood cells, MCV fl, MCH fmol/l, MCHC mmol/l.

DISCUSSION

Total plasma- or serum magnesium concentrations may not adequately reflect body magnesium stores. It was our aim to establish the magnesium content of the red blood cells as an estimation of these body stores. In contrast to the lowering of plasma magnesium, the nadir of red blood cell magnesium was only obtained after 3 weeks of magnesium deprevation. This divergence was also observed in an earlier study, when at the end of the period of magnesium deficiency, serum magnesium was restored after supplementation with MgO [4]. After one week of supplementation the serum magnesium was normal but the erythrocyte content of magnesium was still decreased.

This former study demonstrates that the degree of deficiency in the erythrocytes depends on the duration of the period of deficient diet and probably (no balance studies were performed) on the amount of body magnesium loss. A similar study was recently performed in normal human volunteers by Ryzen et al. [9] In four normal subjects they observed a progressive fall in both serum and red blood cell magnesium during magnesium depletion, with a concomitant rise in retention of parenterally administered magnesium. Only data before and after two and three weeks of magnesium deficiency were available. As was demonstrated for the dogs (vide supra) after 4 weeks of magnesium deprivation, in humans Ryzen et al. [9] observed a decrease of ionized free magnesium in red blood cells, measured with the ^{31}P NMR technique as we did.

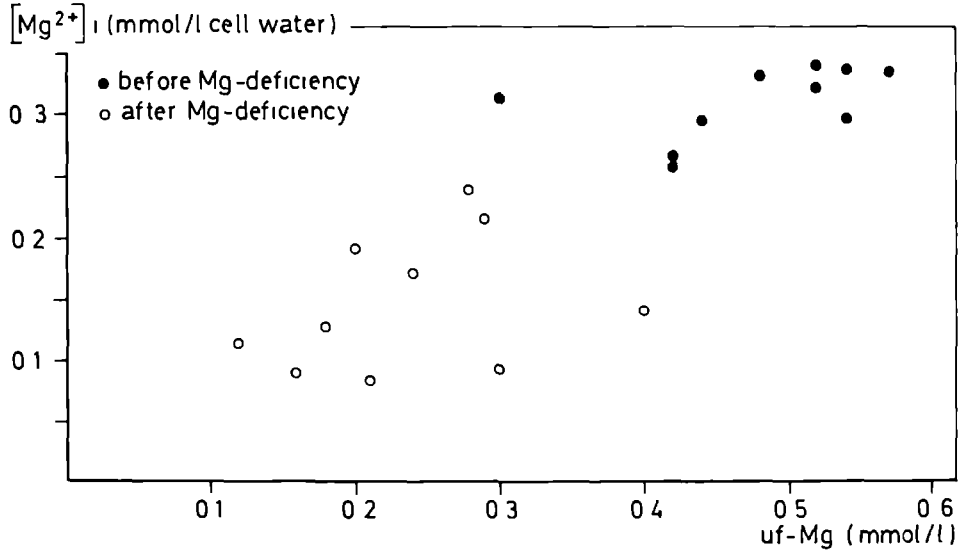

Figure 3 Ionized intracellular magnesium content and plasma ultra filtrable magnesium show an apparent relation

It is striking that the magnesium content of the erythrocytes progressively decreased although these erythrocytes are surrounded by a constant ultrafiltrable and presumably ionized magnesium. The red cell magnesium permeability appears to be very low. Ginsberg et al. [5] could detect no significant uptake of ^{28}Mg by human red blood cells during an incubation period of 24 hours at 37°C. Flatman [2] reported that storage of erythrocytes at 4°C in media containing no magnesium for up to one week leads to little change in red cell magnesium content. Even during a period of magnesium deficiency the red cell ionized magnesium concentration remained below the electrochemical equilibrium This means, that an active magnesium extrusion is still present. As magnesium efflux studies in magnesium loaded red blood cells have demonstrated, this efflux depends for 10% on extracellular sodium and for 90% on

net chloride efflux for charge compensation [7]. A lowering of ionized magnesium outside the erythrocyte, lowers the intracellular concentration of ionized magnesium of the erythrocytes in such a system. The exact mechanism of magnesium transport in human red blood cells is still not definitely established [2].
We can conclude that in dogs with experimental magnesium deficiency, erythrocyte magnesium levels are a better reflection of the body magnesium status than plasma magnesium levels. As the magnesium stores become more depleted during the course of the magnesium deficient diet, the erythrocyte magnesium content progressively decreases while plasma magnesium content reaches its nadir after one week of depletion.

ACKNOWLEDGEMENTS

We thank the members of the Central Animal Laboratory (head: Prof.dr. W.J.I. v.d. Gulden) for their effective support and Ir. Th. de Boo and W. Lemmens for the statistical calculations. This study was supported by the Dutch Kidney Foundation.

REFERENCES

1. D'Costa, M., Cheng, P-T. Ultrafiltrable calcium and magnesium in ultrafiltrates of serum prepared with the Amicon MPS-1 system. Clin.Chem. **29** (1983) 519-522.
2. Flatman, P.W. The control of red cell magnesium. Magnesium Res. **1** (1988) 5-11.
3. Flatman, P.W., Lew, V.L. The magnesium dependence of sodium-pump-mediated sodium-potassium and sodium-sodium exchange in intact human red cells. J.Physiol. **315** (1981) 421-446.
4. Geven, W.B., Vogels-Mentink, G.M., Willems, J.L., De Boo, Th., Lemmens, W. and Monnens, L.A.H. Experimental magnesium depletion in the dog. Influence on the magnesium content of mononuclear cells, erythrocytes, muscle and bone. Mag.-Bull. **10** (1988) 45-50.
5. Ginsburg, S., Smith, J.G., Ginsburg, F.M., Reardon, J.Z., Aikawa, J.K. Magnesium metabolism of human and rabbit erythrocytes. Blood **20** (1962) 722-729.
6. Günther, T. Stoffwechsel und Wirkungen des intrazellulären Magnesiums. J.Clin.Chem.Clin.Biochem. **15** (1977) 433-438.
7. Günther, T. and Vormann, J. Na^+-independent Mg^{2+} efflux from Mg^{2+}-loaded human erythrocytes. FEBS Letters **247** (1989) 181-184.
8. Gupta, R.K., Benovic, J.L., Rose, Z.B. The determination of the free magnesium level in the human red blood cell by ^{31}P NMR. J.Biol.Chem. **253** (1978) 6172-6176.
9. Ryzen, E., Servis, K.L., DeRusso, P., Kershaw, A., Stephan, T., Rude, R.K. Determination of intracellular Free Magnesium by Nuclear Magnestic Resonance in human magnesium deficiency. J.Am.Coll.Nutr. **8** (1989) 580-587.

HUMAN ERYTHROCYTE FREE MAGNESIUM CONCENTRATION: DIFFERENCES BETWEEN ^{31}P NMR AND ZEROPOINT TITRATION

Submitted.

HUMAN ERYTHROCYTE FREE MAGNESIUM CONCENTRATION: DIFFERENCES BETWEEN ^{31}P NMR AND ZEROPOINT TITRATION

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SUMMARY

Intracellular ionized magnesium concentrations $[Mg^{2+}]_i$ were estimated in erythrocytes by ^{31}Phosphorous Nuclear Magnetic Resonance (^{31}P NMR) and zeropoint titration (ZPT) in 14 controls and 7 patients with renal magnesium loss. Total and ultrafiltrable magnesium concentrations in plasma and total magnesium concentrations in erythrocytes were also determined. The mean intracellular ionized magnesium concentration in controls measured by ^{31}P NMR was 0.20 ± 0.03 mmol/L cell water, in contrast to a value of 0.55 ± 0.12 mmol/L cell water obtained by zeropoint titration. Total red blood cell magnesium content measured with the lysate method was 0.63 mmol/L cell water higher than estimated by ^{31}P NMR probably because not all magnesium complexes are fully visible by the NMR technique. We found a positive correlation between plasma ultrafiltrable magnesium and $[Mg^{2+}]_i$ irrespective of the $[Mg^{2+}]_i$-assay used. $[Mg^{2+}]_i$ measured with ^{31}P NMR also correlated with $[Mg^{2+}]_i$ determined by zeropoint titration.
Washing erythrocytes before the zeropoint titration procedure, decreased the ATP content, which could partially be restored by incubating with inosine. The cell water fraction was also lower after the washing procedure. It is concluded that the decrease in cell water and ATP levels in the erythrocytes overestimates $[Mg^{2+}]_i$ when measured with zeropoint titration. Evaluation of influences of dissociation constants used in calculating the ionized and total magnesium concentrations from the NMR results, led us to conclude that a variation in every dissociation constant markedly influences the calculated results of $[Mg^{2+}]_i$. Although absolute values for $[Mg^{2+}]_i$ differ with the assay used, significantly lower values for $[Mg^{2+}]_i$ were found in patients with isolated renal magnesium loss by both methods.

INTRODUCTION

Magnesium is the second most abundant intracellular cation. It is present in bound [MgB] and free $[Mg^{2+}]$ form. Several methods for the measurement of total cellular magnesium content have been described, but intracellular free $[Mg^{2+}]_i$ is physiological relevant and is therefore of special interest. A wide range of $[Mg^{2+}]_i$ has been reported, by several investigators [1] for a number of cell types, using different techniques. A threefold rise in $[Mg^{2+}]_i$ has been reported in deoxygenated red blood cells while total intracellular magnesium content did not change [2]. On the other hand, in hepatocytes $[Mg^{2+}]_i$ appeared to be very sensitive to variations in total magnesium, hence small increases or decreases in total magnesium resulted in large variations of $[Mg^{2+}]_i$ [3].
Differences were also reported between $[Mg^{2+}]_i$ when measured with ^{31}P NMR spectroscopy as developed by Gupta et al. [2] or by zeropoint titration (ZPT) as described by Flatman and Lew [4]. We are not aware, however, of a study in which both techniques are were used simultaneously.
The aim of the present study is to compare ^{31}P NMR being an "indirect" method with the "direct" zeropoint titration method, to measure $[Mg^{2+}]_i$ in human erythrocytes. We

applied both methods to oxygenated erythrocytes from healthy controls and from patients with isolated renal magnesium loss [5].

METHODS

Plasma magnesium was measured by atomic absorption spectrophotometry (Perkin Elmer 5000, Norwalk CT, USA). Ultrafiltrates were obtained after ultrafiltration with Amicon tubes (Amicon Corporation, Danvers, MA, USA) [6]. Erythrocyte total magnesium content was measured in lysed red blood cells as described previously [7], and expressed in mmol/L cell water. Hemoglobin level, hematocrit, number of erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were measured with a H1-jr (Technicon Instruments Corp. Tarrytown, NY, USA).

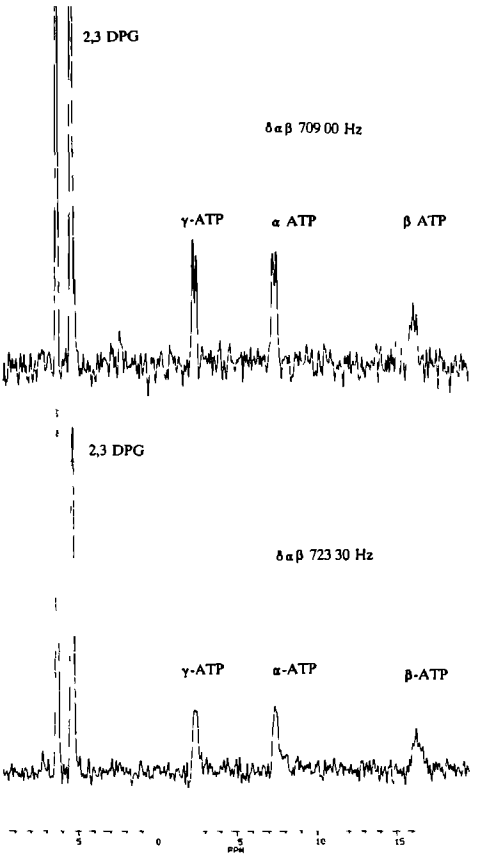

Figure 1. ^{31}P NMR spectra of a control person (upper part) and a renal Mg loss patient (lower panel) show differences in the ß-ATP peak position and δαß. The values for $[Mg^{2+}]_i$ were 0.20 and 0.14 mmol/L cell water respectively.

^{31}P NMR

Ten mL of freshly drawn heparinized venous blood was centrifuged and 10 mmol glucose was added to the plasma. The plasma was mixed with the red blood cells and incubated for 10 min at 20° C. The blood was centrifuged and an erythrocyte-suspension with a hematocrit of ≈ 90 % was obtained. Complete oxygenation was ensured with 95% oxygen and 5% CO_2 in a tonometer (Intrumentation Laboratory, Milano, Italy). The sample was transferred into a 10 mm outer diameter glass NMR tube with a 4 mm outer diameter coaxial insert containing D_2O for deuterium lock.
^{31}P NMR spectra were recorded at 81 MHz and 37° C on a Bruker WM 200 spectrometer (Bruker, Karlruhe, FRG) operating in the Fourier transform mode. Pulses of 55° in combination with gated proton decoupling were applied with a repetition time of 0.78 seconds; the samples were spun at 10 Hz during the entire recording.
The calculations of the concentrations of ATP and 2,3-DPG complexed to Mg^{2+} and/or hemoglobin were done essentially according to Gupta et al. [2] and expressed in mmol/L cell water. The free fraction of ATP not complexed to magnesium (Φ) is calculated from the NMR spectrum by means of the equations listed in Gupta's paper [2], solved with the aid of Newton's iteration method using a program for IBM compatible computers.

$$\Phi = \frac{[ATP]_F + [HbATP]}{[ATP]_T} \quad (1)$$

Where ATP_F is ATP free and ATP_T is ATP total.

$$\Phi = \frac{(\delta^{cell}_{\alpha\beta} - \delta^{MgATP}_{\alpha\beta})}{(\delta^{ATP}_{\alpha\beta} - \delta^{MgATP}_{\alpha\beta})} \quad (2)$$

In which $\delta^{cell}_{\alpha\beta}$ is the measured resonance position of the ß-resonance of ATP with respect to the α-resonance, which has a nearly constant position.

$$[Mg^{2+}]_i = Kd^{MgATP} [(1/\Phi) - 1] \quad (3)$$

Where Kd^{MgATP} is the dissociation constant for MgATP. $Kd^{MgATP} = 3.8 \times 10^{-5}$M at 37°C and pH 7.2 [2].

The total magnesium concentrations are calculated as the sums of the concentrations of all magnesium containing complexes and free magnesium, neglecting the possible amount of magnesium complexed to other red cell constituents.
ATP and 2,3-DPG levels in the red blood cells were determined using comercial kits (Sigma Chemical Co, St Louis).

Zeropoint titration

Ten mL of freshly drawn heparinized venous blood was centrifuged. The plasma was removed and the red blood cells were washed 3 times in medium I (75 mmol/L KCl, 75 mmol/L NaCl, 10 mmol/L Tris-Cl, pH 7.4, 37° C) containing 0.1 mmol/L EGTA to chelate contaminant calcium and extracellular magnesium [4]. The cells were then washed twice in medium I containing 10 μmol/L EGTA and oxygenated with 95% oxygen and 5% CO_2 in a tonometer. Packed cells were then added to the incubation medium (medium I with 10μmol/L EGTA and 10 mmol/L inosine) containing different concentrations of magnesium (hematocrit ±15%). After 10 min incubation at 37° C under constant stirring in plastic vials, samples were taken for the measurement of the initial magnesium content of the cells. At t=0 ionophore A23187 was added to reach a concentration of 10 μmol/L. At t=10 and t=20 min 0.5 mL samples were taken to measure the magnesium content of the cells. Samples were ejected into an Eppendorf microcentrifuge tube containing 0.8 mL icecold inactivation medium I (with 10 μmol/L EGTA) and 0.4 mL di-n-buthylphtalate (density 1.042 g/ml) and immediately centrifuged at 8000 x g for 1 min. The medium and oil were removed, the tube carefully cleaned with a bevel cut disposable "sample cells and chamber cleaner" (Advanced Instruments, Needham Heights, Massachusetts, USA), the cell-pellet lysed in 400 μL distilled water and frozen at -20° C until assay.
At t=10 min besides a sample for the magnesium content of the red blood cells, we took also twice 0.1 mL for measuring the hematocrit, twice 0.1 mL for the water fraction (f_w) and density, and twice 0.4 ml for the chloride distribution ratio (r).

Water fraction(f_w), dry weight(dr.wt) and density(dens.)

The wet and dry weight of 0.1 mL erythrocyte suspension with known hematocrit were measured and the water fraction and density of the red blood cells were calculated. No correction for entrapped water was necessary because the H1-Jr calculates the hematocrit as the number of red blood cells multiplied by their Mean Corpuscular Volume.

Chloride distribution ratio(r)

To an Eppendorf tube containing 0.4 mL cell suspension 50 μL tracer ^{36}Cl (5 μC/mL; Amersham International plc, Amersham, England) was added and incubated at 37° C for 10 min. Then di-n-buthylphtalate (0.4 mL) was added and the sample spun at 8000 x g for 5 min. A 100 μL sample was taken from the supernatant, and the rest was carefully and completely removed before 0.150 mL 5% TCA was added to the cell pellet. ^{36}Cl activities in the supernatant and cell pellet were determined in duplo by liquid scintillation counting (Searle Mark III liquid scintillation counter, Searle Analytic Inc., Des Plaines, Ill., USA).

$$r = [Cl^-]_o / [Cl^-]_i , \quad (4)$$

were $[Cl^-]_o$ and $[Cl^-]_i$ are the ^{36}Cl activities in 100 μL of supernatant and cell water respectively.

Ionized magnesium concentration

The concentration of ionized magnesium in the medium at equilibrium ($[Mg^{2+}]_o$) was extrapolated from the initial and final magnesium concentrations of the red blood cells and the magnesium concentrations in the corresponding medium.
$[Mg^{2+}]_i$ was then calculated according to:

$$[Mg^{2+}]_i = r^2 [Mg^{2+}]_o \quad (5)$$

Statistics

Spearman's rank correlation coefficients were used to detect a possible relation between variables and the signed rank test was taken to test for significant differences between different variables. Results were considered to be significant when $p < 0.05$.

Table 1. Results (mean ± 1 sd) of the different variables in controls and patients with renal magnesium loss.

	Controls		Patients with renal Mg loss		p
n	14		7		
se-Mg mmol/L	0.83	(0.04)	0.46	(0.11)	<0.01
uf-Mg mmol/L	0.57	(0.04)	0.33	(0.09)	<0.01
Lysate:					
$[MgT]_i$ mmol/L cell w.	3.00	(0.38)	2.77	(0.14)	ns
^{31}P NMR					
$[MgT]_i$ mmol/L cell w.	2.37	(0.22)	1.94	(0.17)	<0.01
$[Mg^{2+}]_i$ mmol/L cell w.	0.20	(0.03)	0.13	(0.02)	<0.01
ZeroPoint Titration					
n	11		6		
$[Mg^{2+}]_i$ mmol/L cell w.	0.55	(0.12)	0.36	(0.03)	<0.01

RESULTS

In table 1 the relevant data obtained from the blood of controls and patients with renal magnesium loss are listed. In comparison to controls, patients have significantly lower values for plasma, ultrafiltrable and $[Mg^{2+}]_i$, measured either with NMR and ZPT, than controls. In patients with renal magnesium loss Φ is higher than in controls; 0.265 respectively 0.193, so relatively more magnesium is complexed with

ATP. No differences between the two groups were found for ATP, DPG, water fraction, chloride distribution ratio and density. The total erythrocyte magnesium contents measured in the lysate by atomic absorption spectrometry were not lower in patients compared with controls. However, when the total erythrocyte magnesium content was measured by ^{31}P NMR, the patients had significant lower values than the controls.

Figure 1 shows the ^{31}P NMR spectra of one control and one patient with renal magnesium loss. A difference in the ß-ATP peak position is noticed, resulting in dαß being 709.00 Hz in the control and 723.30 Hz in the patient with renal magnesium loss, whereas $[Mg^{2+}]_i$ was 0.20 and 0.14 mmol/L cell water respectively.

In figure 2 the relation between plasma ultrafiltrable magnesium and NMR-$[Mg^{2+}]_i$ (upper panel) and ZPT-$[Mg^{2+}]_i$ (lower panel) is shown. Plasma ultrafiltable Mg is positive correlated with NMR-$[Mg^{2+}]_i$ ($r = 0.71$, $p < 0.01$) as well as ZPT-$[Mg^{2+}]_i$ ($r = 0.61$ and $p < 0.01$).

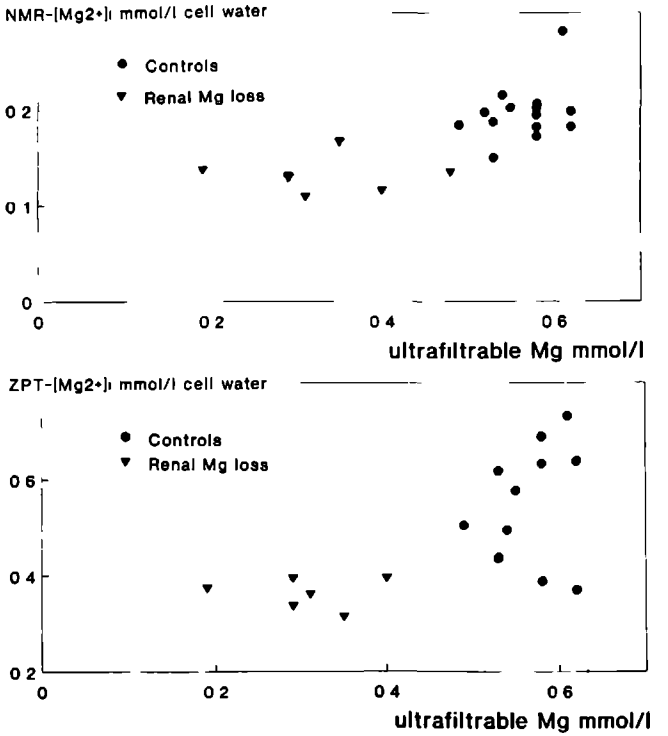

Figure 2. Plasma ultrafiltrable magnesium and NMR-$[Mg^{2+}]_i$ show a good correlation ($r = 0.71$, $p < 0.01$)(upper panel), whereas ZPT-$[Mg^{2+}]_i$ and plasma ultrafiltrable Mg have also a good correlation ($r = 0.61$, $p < 0.01$)(lower panel).

Total erythrocyte magnesium concentration ($[MgT]_i$) shows a significant correlation with $[Mg^{2+}]_i$ obtained with both methods (figure 3). $[MgT]_i$ correlates ($r = 0.63$, $p < 0.01$) with NMR-$[Mg^{2+}]_i$ as well as with ZPT-$[Mg^{2+}]_i$ ($r = 0.66$, $p < 0.01$). In twelve out of fourteen controls NMR-$[Mg^{2+}]_i$ (= 0.20 mmol/L cell water) is nearly constant while total magnesium varies between ± 2.5 and 3.7 mmol/L cell water (figure 3a).
When we compare the results obtained by NMR and ZPT we observe that NMR-$[Mg^{2+}]_i$ remains nearly constant for a wide range of ZPT-$[Mg^{2+}]_i$ values (figure 4) in controls and at a lower level also in samples taken from patients with renal magnesium loss. NMR-$[Mg^{2+}]_i$ and ZPT-$[Mg^{2+}]_i$ correlate ($r = 0.58$, $p < 0.01$) when the samples of controls and patients are taken together.
In order to find an explanation for the considerable difference in $[Mg^{2+}]_i$ values described by the two methods, we investigated the differences in ATP, DPG, the water fraction and dry weight before and after the washing procedure in the ZPT experiments. Total erythrocyte magnesium and DPG (not shown) did not change, but ATP decreased considerably during washing and was partly restored during incubation with inosine prior to the addition of the ionophore. The final decrease in ATP concentration ranged between 6 to 15%, compared to the situation before washing.

Table 2. Water fraction and dry weight before (I) and after (II) washing and then incubation in buffer (III) containing different magnesium concentrations.

		n	fH_2O	p	dry weight	p
I	before washing	8	0.722 (0.029)		0.406 (0.020)	
II	after washing	7	0.673 (0.042)	<0.02	0.465 (0.036)	ns
incubation in						
III	Mg 0.3 mmol/L	8	0.666 (0.065)	ns	0.487 (0.032)	<0.05
	Mg 0.1 mmol/L	5	0.655 (0.088)	ns	0.505 (0.014)	ns

The water fraction and dry weight were measured before, immediately after washing and after 10 min incubation with 0.1 or 0.3 mmol/L magnesium and inosine (10mmol/L) in the presence of the ionophore. The water fraction decreased after the washing and incubation procedure (table 2). Dry weight was the same before and after the ZPT-washing procedure.
To test the influence of dissociation constants on the results, obtained from the ^{31}P shifts by means of in the NMR technique, a value was drawn at random for every dissociation constant out of all values between the mean ± 2 sd. Then NMR-$[Mg^{2+}]_i$ and NMR-$[MgT]_i$ were calculated. This procedure was repeated 1000 times for every control and patient. From this approach it followed that NMR-$[Mg^{2+}]_i$ is 0.19 ± 0.08 in controls and 0.13 ± 0.04 in renal magnesium loss patients, whereas NMR-$[MgT]_i$ is 2.36 ± 0.25 and 1.92 ± 0.17 respectively.

DISCUSSION

For the determination of $[Mg^{2+}]_i$ in erythrocytes no "golden standard" is available and the two methods, ^{31}P NMR and zeropoint titration, lead to different $[Mg^{2+}]_i$ values.

^{31}P NMR

The mean [MgT]i, the result of the sum of the concentrations of magnesium containing complexes and the free magnesium concentration, was approximately 0.6 mmol/L cell water lower when determined by the NMR than measured via the lysate method.

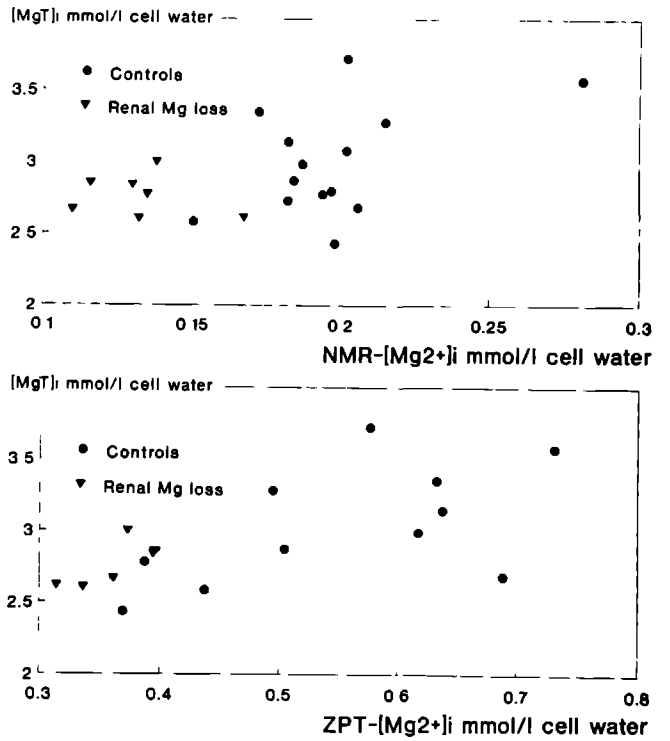

Figure 3. Total erythrocyte magnesium content and $[Mg2+]_i$ are significantly correlated, although $[Mg^{2+}]_i$ shows little variations while $[MgT]_i$ varies from 2.5 to 3.7 mmol/L cell water.

It is suggested that this difference of approximately 25% might be caused by binding of magnesium to cell membranes [8]. In our calculations of the [MgT]i from the NMR data all possible complexes considered by Gupta [2] were accounted for. This includes the hemoglobin ATP complex (HbO_2[ATP]). The dissociation constant of the latter

complex may vary considerably, i.e. Kd = 3.43 ± 0.85mmol [2]. In addition, not all ATP may be available for binding to magnesium, a situation not recognized in the calculations. After critical evaluation [9,10] of the dissociation constant for Mg-ATP[2], it was concluded that Kd^{MgATP}, as used in this paper, is correct. Recently, Petersen et al. [11] stated that NMR-$[Mg^{2+}]_i$ measurements derived solely from the separation of the α- and ß-ATP peaks do not lead to 'a true measurement of' intracellular free magnesium levels and that the concentrations of the magnesium ligands ATP and 2,3-DPG must be taken into account [11].
We found a considerable range of NMR-$[Mg^{2+}]_i$, but NMR-$[MgT]_i$ showed less variation. The observed spreading in NMR-$[Mg^{2+}]_i$ is not caused by variations in oxygenation and pH [11]. Full oxygenation (SaO_2 > 98%) was achieved and the pH of the blood sample was 7.38 ± 0.01.

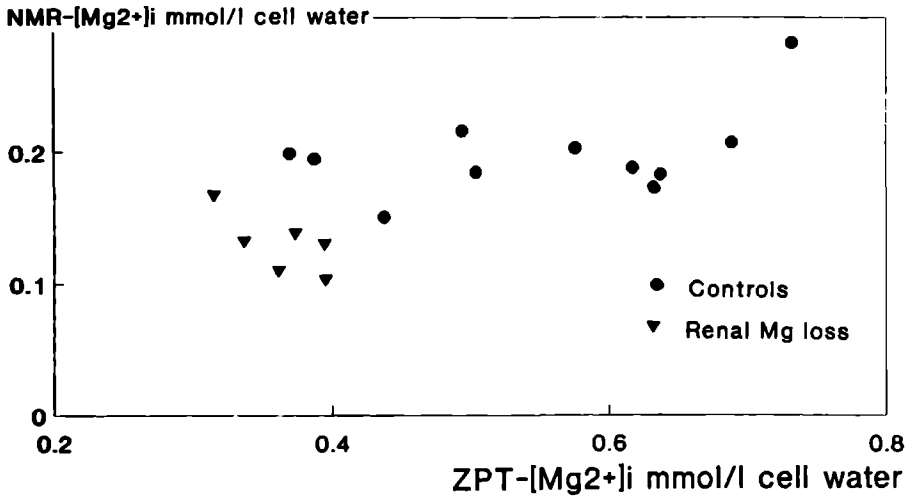

Figure 4. ^{31}P NMR and Zeropoint titration measurement of $[Mg^{2+}]_i$ show a significant correlation; r=0.58, p<0.02.

Zero point titration

Values of $[Mg^{2+}]_i$ obtained with zeropoint titration varied from 0.4 mmol/L cell water[4] to 0.75 mmol/L cells [12] corresponding to 1.13 mmol/L cell water, assuming a cell water fraction of 0.66. However in Wehlings study $[Mg^{2+}]_i$ was estimated from the amount of magnesium released from magnesium loaded erythrocytes [12].
In our study water fraction and chloride distribution ratio were similar to the values reported by Flatman et al. [4] but mean ZPT-$[Mg^{2+}]_i$ was ± 0.15 mmol/L cell water higher in our study. Most likely, the limited number of donors (n=2) investigated by Flatman et al. may explain this difference.

Differences between ^{31}P NMR and Zero point titration

Although our results derived with both methods are close to the reported normal values, the two techniques yield a considerable mean difference of 0.35 mmol/L cell water in $[Mg^{2+}]_i$ in controls. Bock et al. [13] suggested that the washing step used before application of the null point titration procedure might alter $[Mg^{2+}]_i$ and reported an increase of $[Mg^{2+}]_i$ after washing stored red blood cells in buffer. The decrease in erythrocyte ATP found after washing red blood cells for zero point titration and the partly restoration of the ATP level during the following incubation in a magnesium-inosine-ionophore containing buffer might be a possible source of the difference in $[Mg^{2+}]_i$. Flatman and Lew [4] pointed to a nearly ten-fold increase of $[Mg^{2+}]_i$ when the ATP level was lowered from normal (1.66) to 0.2 mmol/L cell water. So a final decrease in the intracellular ATP level of 6 - 15% that we found in erythrocytes used for null point titration might be, at least partly, responsible for the difference in $[Mg^{2+}]_i$ found between ^{31}P NMR and zeropoint titration. Further investigations are necessary to prove this suggestion.
The changes in water fraction and dry weight which observed to occur during the washing procedure and the further changes in dry weight during the null point titration in a medium containing higher magnesium concentrations, indicate changes in cell volume and results in an increase of $[Mg^{2+}]_i$ measured with ZPT of ± 10%.

Ultrafiltrable magnesium

In controls the ionized magnesium concentration in the red blood cell is normally below the electrochemical equilibrium concentration [14]. The same applies to our patients with isolated renal magnesium loss. An active magnesium extrusion mechanism is present which was shown to be dependent on extracellular sodium for 10% and on net chloride efflux for charge compensation for 90% [15]. Lower extracellular magnesium concentrations lead to lower intracellular ionized magnesium concentrations in magnesium loaded red blood cells [15]. It is striking that the intracellular ionized magnesium concentrations as measured by zeropoint titration approaches the value of ultrafiltrable Mg is plasma. However, the exact mechanism of magnesium transport and homeostasis in human erythrocytes is not jet established [14].
The zeropoint titration method should be prefered because it is a direct method. The interpretation of the ^{31}P NMR results is dependent on the available K-values; deviations in the equilibrium constants or in the availability of the substrate may result in incorrect values of the calculated magnesium concentration. Although the both methods lead to different results, significant differences between controls and patients with renal magnesium loss are obtained independent of the method used.

ACKNOWLEDGEMENTS

We thank W. Lemmens and Th. de Boo from the department of medical statistics for their effective advice and help. This study was supported by a grant from the Dutch Kidney Foundation.

REFERENCES

1. Alvares-Leefmans, F.J., Giraldez, F., Gamino, S.M. Intracellular free magnesium in excitable cells: its measurement and its biologic significance. Can. J. Physiol. Pharmacol. 1987;65:915-25.
2. Gupta, R.K., Benovic, J.L., Rose, Z.B. The determination of the free magnesium level in the human red blood cell by ^{31}P NMR. J. Biol. Chem. 1978;253:6172-6.
3. Corkey, B.E., Duszynski, J., Rich, T.L., Matschinsky, B., Williamson, J.R. Regulation of free and bound magnesium in rat hepatocytes and isolated mitochondria. J. Biol. Chem. 1986;261:2567-74.
4. Flatman, P.W., Lew, J.L. Magnesium buffering in intact human red blood cells measured using the ionophore A23187. J. Physiol. 1980;305:13-30.
5. Geven, W.B., Monnens, L.A.H., Willems, J.L., Buijs, W., Ter Haar, B. Renal magnesium wasting in two families with autosomal dominant inheritance. Kidney Intern. 1987;31:1140-4.
6. D'Costa, M., Cheng, P-T. Ultrafiltrable calcium and magnesium in ultrafiltrates of serum prepared with the Amicon MPS-1 system. Clin.Chem. 1983;29:519-22.
7. Geven, W.B., Vogels-Mentink, G.M., Willems, J.L., De Boo, Th., Lemmens, W., Monnens, L.A.H. Experimental magnesium depletion in the dog. Influence on the magnesium content of mononuclear cells, erythrocytes, muscle and bone. Mag.-Bull. 1988;10:45-50.
8. Fujii, T., Sato, T., Hanzawa, T. Calcium and magnesium contents of mammalian erythrocyte membranes. Chem. Pharm. Bull. 1973;21:171-5.
9. Garfinkel, L., Garfinkel, D. Calculation of free-Mg^{2+} concentration in adenosine 5'-triphosphate containing solutions in vitro and in vivo. Biochemistry. 1984;23:3547-52.
10. Gupta, R.K., Gupta, P., Yushok, W.D., Rose, Z.B. On the noninvasive measurement of intracellular free magnesium by ^{31}P NMR spectroscopy. Physiol. Chem. Phys. Med. NMR. 1983;15:265-80.
11. Petersen, A., Kristensen, S.R., Jacobsen, J.P., Horder, M. ^{31}P-NMR measurements of ATP, ADP, 2,3-diphosphoglycerate and Mg^{2+} in human erythrocytes. Biochim. Biophys. Acta. 1990;1035:169-74.
12. Wehling, M., Theisen, K. Magnesium release from red blood cells of hypertensive man by the ionophore A23187. Magnesium 1988;7:44-8.
13. Bock, J.L., Yusuf, Y. Further studies on alterations in magnesium binding during cold storage of erythrocytes. Biochim. Biophys. Acta. 1988;941:225-31.
14. Flatman, P.W. The control of red cell magnesium. Magnesium Res. 1988;1:5-11.
15. Günther, T. and Vormann, J. Na^+-independent Mg^{2+} efflux from Mg^{2+}-loaded human erythrocytes. FEBS Letters 1989;247:181-4.

Chapter 9

STUDY OF PATHOPHYSIOLOGY OF BARTTER/GITELMAN'S SYNDROME

Attempt of classification - Role of renal magnesium depletion

Submitted.

STUDY OF PATHOPHYSIOLOGY OF BARTTER/GITELMAN'S SYNDROME

Attempt of classification - Role of renal magnesium depletion

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SUMMARY

Based on the serum magnesium level, the urinary excretion of calcium, the concentrating ability and the glomerular filtration rate it is possible to make a distinction between Bartter's syndrome, a defect localized in the medullary part the thick ascending limb of the loop of Henle and Gitelman's syndrome, a defect localized in the distal nephron after the medullary part of the ascending limb of the loop of Henle. A few patients, however, cannot be classified. From the fifteen investigated patients seven had a lowered serum magnesium level. From this seven five had also a slightly reduced content of magnesium in the erythrocyte. The magnesium content of the mononuclear cells expressed as μg/protein or μg/DNA was normal in all patients. A pronounced magnesium deficiency was apparently not present in these group of patients.

INTRODUCTION

Bartter's syndrome is characterized by chronic hypokalemia due to renal potassium wasting accompanied by mild to moderate alkalosis and hypochloremia. Defective chloride transport in the thick ascending limb of loop of Henle is considered to be the primary cause of Bartter's syndrome. The increased prostaglandin production and hyperreninemia are thought to be secondary as it is also observed in pseudo-Bartter's syndrome (e.g. habitual vomiting). A defect in the tubular reabsorption of magnesium associated with renal potassium loss (Gitelman's syndrome) can be distinguished from Bartter's syndrome by the milder symptoms, a normal diluting ability and relatively intact concentrating ability according to Gill et al. [1]. As described further on in this paper new criteria will be formulated for both syndromes. In a study of an infant with the severe type of Bartter's syndrome Mach et al. [2] observed a lowered content of muscle magnesium although the serum magnesium level was normal. After the introduction of oral magnesium therapy, there was marked improvement in muscle strength; the intramuscular concentration of magnesium increased to a normal level. In four adult patients with a decreased serum concentration of potassium and magnesium, a normal amount of magnesium was observed in muscle tissue [3]. In thirteen children and two adults with Bartter/Gitelman's syndrome, serum magnesium and urinary magnesium excretion were determined together with the intracellular content of magnesium in the erythrocytes and mononuclear cells.
Magnesium depletion appeared to be absent in mononuclear cells in both syndromes. Five out of the seven patients with a lowered serum magnesium had also a lowered magnesium content of the erythrocytes. Serum magnesium and the magnesium content of the erythrocytes were significantly correlated.

PATIENTS AND METHODS

In table I the most relevant blood (plasma, serum) values are presented of the

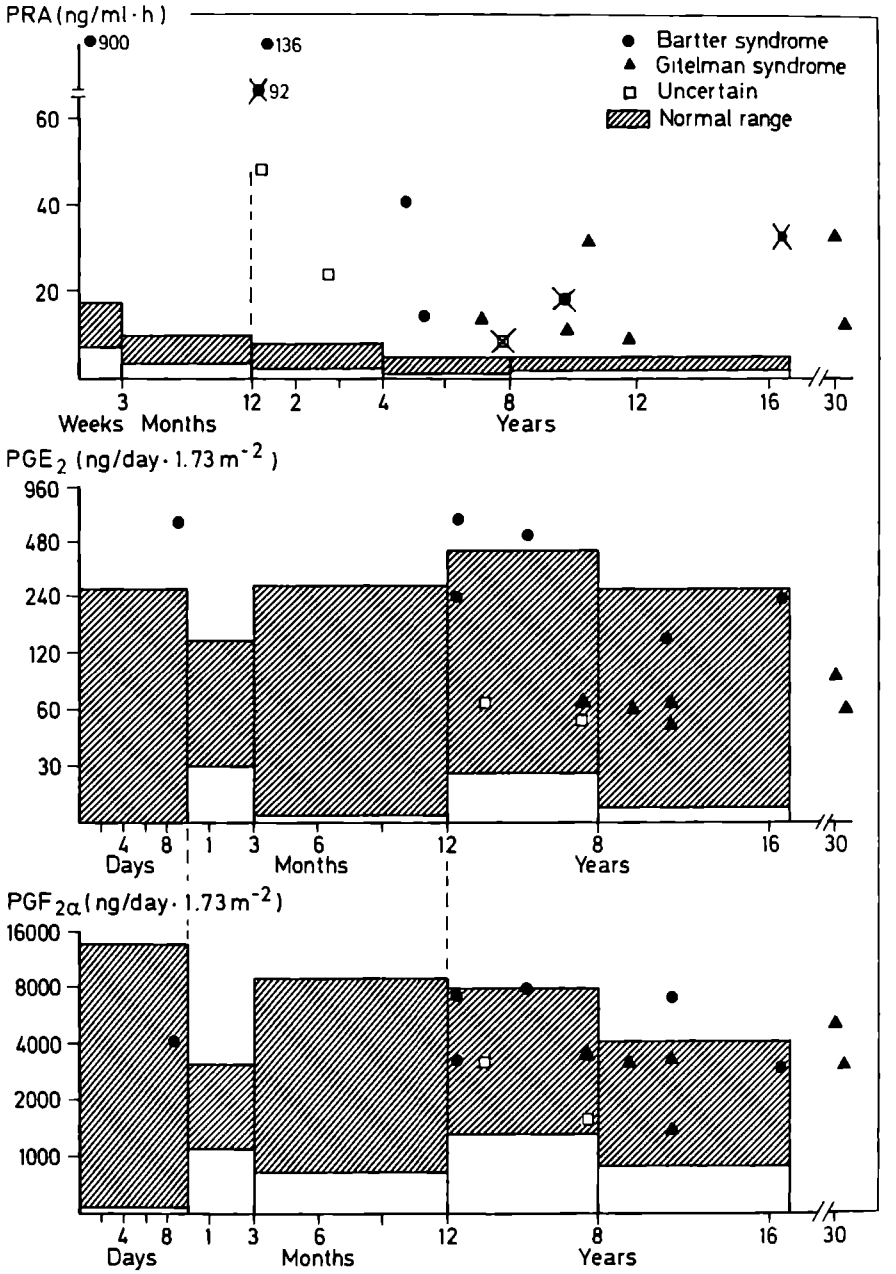

Figure 1. Plasma renin activity and urinary excretion of prostaglandin E_2 and $PGF_{2\alpha}$ in all patients according to age.
X indicates clearance of creatinine < 80 ml/min.1.73 m^2.

Table 1. Relevant blood values in the fifteen investigated patients.

Initials	Age at the time of investigation:	Na	K	Cl	HCO_3	pH	BE	pCO_2	Mg	Ca	plasma renin activity	classification
	years	mmol/l					mmol/l	kPa	mmol/l		ng/ml.hour	
1. B.B.	1 2/12	136	2.7	100	26,0	7,39	0,9	6,0	1,00	2,63	91,9	Bartter
2. B.R.	1 4/12	138	3,4	81	33,7	7,50	18,5	5,7	0,83	2,81	48,3	?
3. A.T.	9 4/12	141	2,7	99	28,5	7,47	5,3	5,3	0,91	2,50	19,0	Bartter
4. G.M.	17 4/12	138	3,1	-	27,5	7,38	1,8	6,4	0,89	2,52	33,0	Bartter
5. N.J.	1 4/12	138	3,0	102	23,9	7,46	11,2	4,7	1,03	2,36	136,0	Bartter
6. M.L.	7 8/12	138	3,6	105	23,6	7,43	0,2	4,9	0,77	2,37	9,1	?
7. A.S.	2 8/12	137	3,5	100	26,6	7,39	1,5	6,1	0,78	2,44	23,0	?
8. Y.C.	3 weeks	118	2,7	67	29,9	7,47	6,3	5,6	0,86	2,30	900	Bartter
9. A.B.	4 7/12	141	3,8	101	23,6	7,44	-1,0	4,8	0,88	2,55	40,7	Bartter
10. J.v.d.R.	7 4/12	145	2,3	103	26,2	7,39	1,5	5,7	0,69	2,39	14,6	Gitelman
11. C.v.d.H.	11 5/12	138	2,6	93	31,3	7,42	5,9	6,7	0,62	2,52	31,8	Gitelman
12. Y.v.d.H	30	138	2.5	94	27.5	7.46	4.4	5.1	0.54	2.76	32,2	Gitelman
13. M.V.	11 4/12	141	2,9	96	29,5	7,38	3,4	6,9	0,58	2,40	9,5	Gitelman
14. S.V.	9 8/12	143	2,7	97	26,5	7,42	2,4	5,6	0,68	2,53	11,3	Gitelman
15. v.d.H-H.	31	139	2,1	95	27,7	7,51	5,9	4,7	0,58	2,59	11,8	Gitelman

Patients 11-12 and 13-14 are siblings.

Table 2. Urinary excretion of prostaglandins, magnesium and calcium in all patients.

Initials	Prostaglandin excretion				Mg and Ca excretion	
	PGE_2 ng/day.1,73 m2 SA	$PGF_{2\alpha}$	6-Keto-$PGF_{1\alpha}$	TXB_2	Mg mg/kg day	Ca
1. B.B.	244 N	3685 N	1200 N	496 N	2,9	5,4
2. B.R.	-	-	-	-	3.6	0,4
3. A.T.	155 N	7532 ↑	3683 ↑	785 ↑	1,3	3,7
4. G.M.	238 N	3165 N	2462 ↑	403 N	-	8,6
5. N.J.	667 ↑	7752 N	2439 N	579 N	3,8	9,1
6. M.L.	59,3 N	1721 N	859 N	297 N	3,3	0,3
7. A.S.	70,3 N	3428 N	1725 N	382 N	3,0	0,4
8. Y.C.	670 ↑	4606 ↑	1536 ↑	512 ↑	3,6	2,2
9. A.B.	514 ↑	8823 ↑	2075 N	373 N	5,0	15,0
10. J.v.d.R.	69,2 N	3639 N	352 N	234 N	3,5	1,5
11. C.v.d.H.	69,0 N	3496 N	1522 N	225 N	1,0	0,2
12. Y.v.d.H	62.0 N	3326 N	2609 N	734 N	-	0.3
13. M.V.	136 N	3830 N	1600 N	568 N	2,1	0,3
14. S.V.	62,0 N	3404 N	1628 N	351 N	3,7	3,9
15. v.d.H-H.	92 N	5217 ↑	2699 N	1139 N	2.1	0.4

↑ increased; N normal values from [5,6].

normal values according to Paunier in infants and children (1 month-14 years) [11].
Ca 1.1 - 7.4 mg/kg.day
Mg 0.9 - 5.2 mg/kg.day
normal values according to Ghazali and Barratt (1-11 years old) [12].
Ca 2.38 ± 0.66 SD mg/kg.day
Mg 2.82 ± 0.78 SD mg/kg.day

patients before any form of treatment was given. All patients showed hypokalemic alkalosis and had a distinctly raised plasma renin activity (Fig. 1). Serum magnesium (normal values 0.70-1.04 mmol/l) was lowered in patient 10 to 15.
The urinary excretion of the different prostaglandins PGE_2, $PGF_{2\alpha}$, 6 Keto-$PGF_{1\alpha}$ and TXB_2 as well as the urinary excretion of magnesium and calcium is given in table II. Only in the minority of the patients an increased excretion of some of the prostaglandins was noted (Fig. 1). The urinary Mg excretion was normal in all patients. In 7 patients the calcium excretion was lower than 1,1 mg/kg.day, in 3 patients the calcium excretion was higher than 7 mg/kg.day.

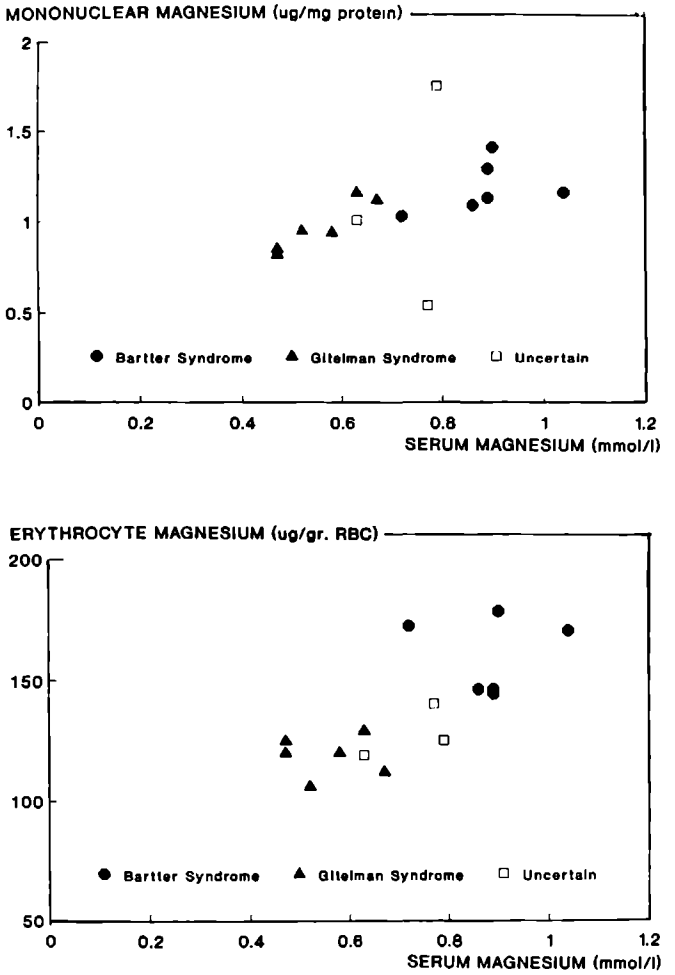

Figure 2. Relations between the serum magnesium level and the cellular content of magnesium in erythrocytes and mononuclear cells shows a significant positive correlation ($r=0.76$, $p>0.01$ and $r=0.71$, $p>0.01$ respectively).

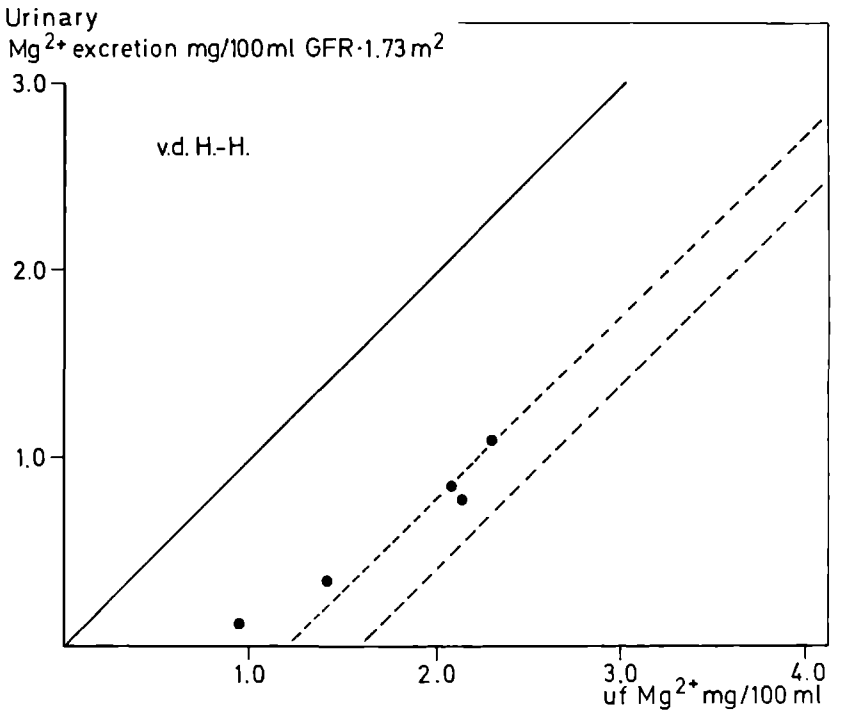

Figure 3. Ultrafiltrable serum Mg concentrations are plotted against urinary Mg excretion. The area between the dotted lines represents the 95% confidence limits according to Rude et al. [10].

The main results of the hypotonic saline infusion test are shown in table III. The distal chloride delivery was increased in two patients (5,7) (omitting the patients with diminished GFR). The percentage of the distal chloride load being reabsorbed distally was lowered in all patients without exception. This is in agreement with the limited dilution (Uosmol) obtained during the experimental procedure. The chloride data are preferred to the sodium data as these data do not take into account the sodium reabsorbed distally by exchange with potassium and hydrogen ion. In table III the values of the endogenous clearance of creatinine together with urinary concentrating capacity obtained after DDAVP are also presented. The clearance of creatinine was lowered in 5 patients (1, 3, 4, 6, 8). In five of the patients (number 7-10-11-12-15) a normal concentrating capacity was measured. A slightly decreased capacity was present in patients 6-13-14.

The plasma renin activity [4], the prostaglandin excretion [5,6] and the DDAVP test [7] were performed according to previously reported methods. The study of the fractional clearances during hypotonic saline diuresis were based on the findings of Rodriguez-Soriano et al. [8]. The methods for extra- and intracellular magnesium are described earlier [9]. The renal magnesium threshold and tubular maximum for magnesium were done according to Rude et al. [10]. The normal values for the urinary excretion of calcium and magnesium are obtained from Paunier et al. [11] and Ghazali and Barratt [12].

Table 3. Results of the hypotonic saline perfusion test, creatinine clearance and DDAVP test.

Initials	Uosmol mosmol/kg	Ck ml/100ml GFR	$C_{H2O} + C_{Cl}$ ml/100ml GFR	$\frac{C_{H2O}}{C_{H2O} + C_{Cl}} 100\%$	Clearance of creatinine ml/min.1,73 m2	Concentrating capacity mosmol/kg
1. B.B.	186	97	20.6	31.6	59	377
2. B.R.	-	-	-	-	86	534
3. A.T.	111	41.9	-	55.0	70	445
4. G.M.	107	155.0	39.9	60.9	42	171
5. N.J.	146	128.3	25.2	46.9	99	509
6. M.L.	113	24.8	16.1	56.4	41	748
7. A.S.	121	46.5	36.2	50.3	85	1166
8. Y.C.	-	-	-	-	18	300
9. A.B.	179	15.5	2.3	51.9	127	294
10. J.v.d.R.	73	34.1	16.7	70.9	80	1015
11. C.v.d.H.	121	31.7	8.4	63.5	135	1070
12. Y.v.d.H.	-	-	-	-	116	980
13. S.V.	113	83.2	10.1	66.6	122	719
14. M.V.	103	52.4	9.1	68.8	120	802
15. M.v.H.-H.	93	21.0	6.0	72.8	150	914
Normal values	54.1 ± 13.3	12.9 ± 5.2	15.9 ± 2.6	86.7 ± 4.1	80-120	≥ 807

Statistical methods

We used Spearman's rank correlation coefficients to detect relationships between different variables. Results were considered to be significant when $p<0.05$.

RESULTS

Six of the 15 studied patients had permanently a lowered serum magnesium level. These six patients had a normal glomerular filtration rate and a normal or slightly decreased concentration ability.

One of other patients (number 2) had initially a normal serum magnesium level. A significant positive correlation was noted between the serum magnesium and potassium level ($r=0.76$, $p<0.01$).

The results of the intracellular magnesium and potassium levels in mononuclear cells and erythrocytes are shown in table IV. Almost all values for intracellular magnesium in mononuclear cells were in the normal range. In these cells a positive correlation existed between the serum magnesium level and the concentration of magnesium only when the magnesium concentration was expressed in terms of cellular protein content ($r= 0,71$), $p < 0,01$) (fig. 2).

Five patients had a lowered magnesium content in the erythrocytes. There was a significant positive correlation between the serum magnesium level and the intracellular concentration of magnesium in the erythrocytes ($r= 0,76$, $p < 0.01$)(fig. 2).

In two patients (number 11, 15) a lowered renal magnesium threshold was found together with a normal tubular maximum for magnesium (Fig. 3).

DISCUSSION

For the interpretation of the metabolic studies an adequate classification is required of the familial hypokalemia due to renal potassium loss. Bartter's syndrome is frequently confused with the syndrome of hypokalemia/hypomagnesemia first described by Gitelman et al. [13].

Classification of the patients

Six out of 15 patients had a lowered serum magnesium level with a normal magnesium excretion pointing to a defect in magnesium reabsorption (number 10-15). These patients were asymptomatic with the exception of occasionally episodes of muscular weakness and tetany. Patient number 12, however, was severely handicapped by her disorder and unable to perform even light domestical work due to muscular weakness. After treatment with magnesium suppletion and amiloride a normal life became possible.

In the six patients with Gitelman's syndrome the same abnormalities as in the 9 other patients were found for plasma renin activity, prostaglandin excretion, plasma potassium, chloride, and metabolic alkalosis. The distal chloride resorption defect

Table 4. Results of the intracellular concentration of Magnesium and Potassium together with main serum electrolytes at the time of investigation.

Initials	Serum Mg	Na	K	Cl	Ca	mo-Mg			mo-K			mo-prot	mo-DNA	er-Mg	er-K
	mmol/l					fg/cell	µg/mg prot	µg/mg DNA	fg/cell	µg/mg prot	µg/mg DNA	fg/cell	pg/cell	mg/gr RBC	mg/gr RBC
1. B.B.	0,90	136	3,6	105	2,50	124	1.41	13.7	1459	16.6	162	0,09	9,0	178	9.5
2. B.R.	0,63	142	3,3	91	2,60	132	1.01	19.8	1684	12.9	253	0,131	6,7	119	8.4
3. A.T.	0,72	140	3,2	96	2,56	112	1.03	14.8	1553	14.3	206	0,109	7,5	172	10.0
4. G.M.	0,89	139	3,4	-	2,52	141	1.13	15.4	1714	13.7	186	0,125	9,2	144	9.1
5. N.J.	0,89	144	3,4	96	2,61	107	1.29	11.1	1326	16.0	138	0,083	9,6	146	9.0
6. M.L.	0,77	138	3,4	103	2,45	53	0,54	5,7	1264	12.9	135	0,098	9,3	140	-
7. A.S.	0,79	140	3,4	101	2,44	104	1,75	-	-	-	-	0,099	-	125	-
8. Y.C.	1,04	134	3,7	92	2,72	110	1.16	11.9	1482	15.6	161	0,095	9,1	170	9.5
9. A.B.	0,86	140	3,6	102	2,50	102	1.09	11.4	1229	13.1	137	0,094	9,0	146	9.4
10. J.vd.R.	0,63	142	3,2	103	2,39	55	1.16	7.8	896	18.8	127	0,050	7,1	129	8.7
11. C.v.d.H.	0,47	141	2,4	90	2,50	91	0.83	12.3	1073	9.8	146	0,110	7,3	120	9.6
12. Y.v.d.H.	0,47	-	2,5	-	-	96	0.84	12.9	1101	9.6	147	0,115	7,5	125	8.1
13. M.V.	0,52	137	2,6	102	2,50	109	0.95	10.4	1563	13.7	149	0,110	10,5	106	8.8
14. S.V.	0,67	137	2,5	96	2,57	115	1.12	11.2	1198	11.6	116	0,100	10,3	112	7.3
15. v.d.H-H.	0,58	139	2,1	95	2,46	95	0.94	11.7	1302	13.0	161	0.100	8,1	120	8.5
range of normal values						71-156	0.53-1.83	2.9-23.3	787-2668	8.19-20.7	104-334	0.054-0.205	5.2-11.4	125-193	8.2-11.7

mo = mononuclear cells.
er = erythrocytes.

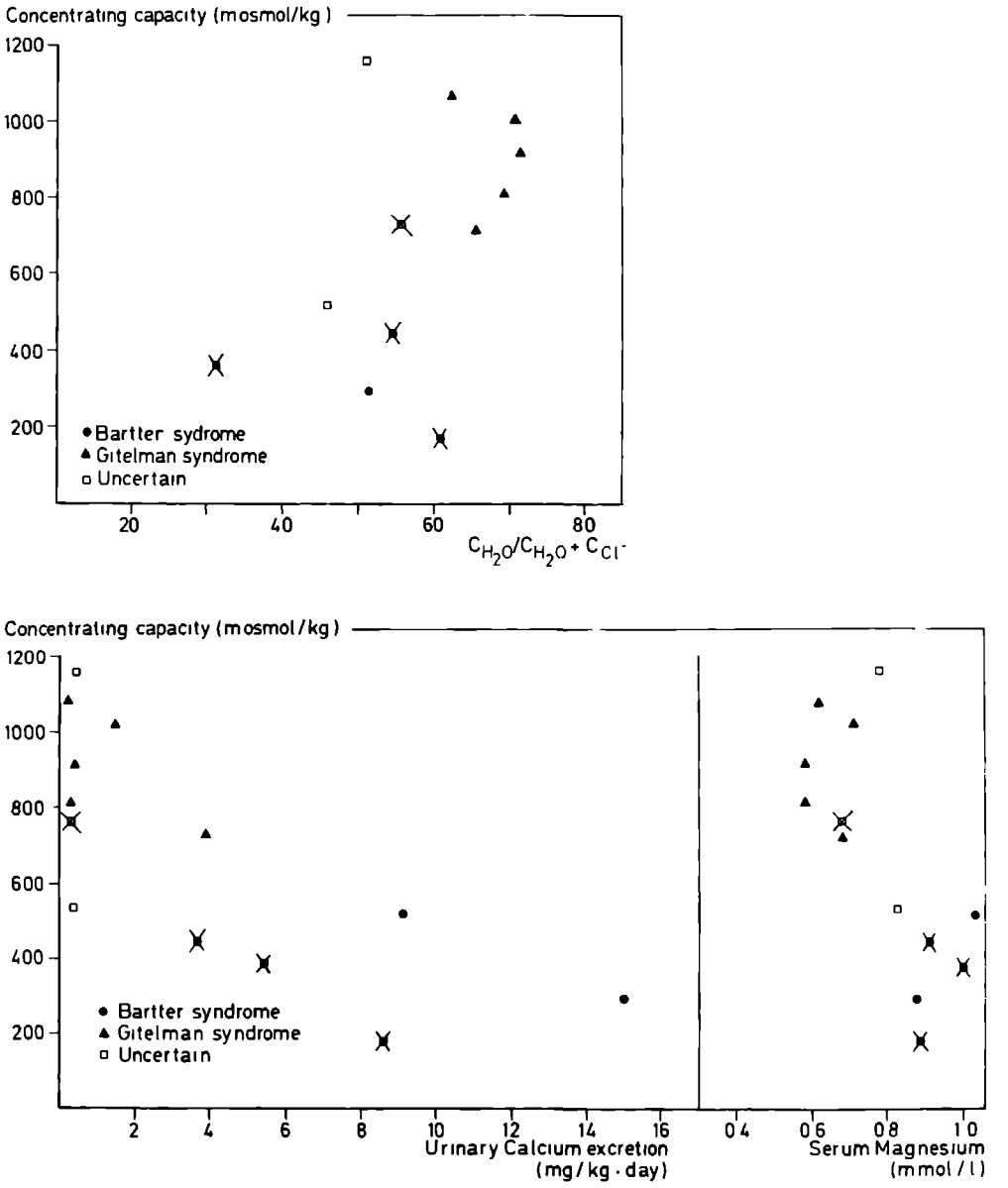

Figure 4. The concentrating capacity and the distal chloride transport measured during the hypotonic saline infusion test were not significantly correlated (r=0.41, p=0.18) (upper panel).
Significant correlation was present between the concentrating capacity obtained after DDAVP and the urinary calcium excretion (r=-0.76, p<0.01) and serum magnesium concentration (r=-0.72, p<0.01) respectively (lower panel).
X indicates clearance of creatinine < 80 ml/min.1.73 m^2.

was milder. All six patients had a normal or slightly decreased concentrating capacity as measured by the DDAVP test. The urinary calcium excretion was either decreased or normal. It is striking that in one of siblings (patient number 13) the urinary calcium excretion was lowered while in the other sibling (14) the calcium excretion was in the normal range. In both siblings the calcium excretion was repeatedly measured. All six patients had a normal glomerular filtration rate.
Based on the criteria of distinctly decreased concentrating capacity, a normal or increased calcium excretion, a normal or decreased glomerular filtration rate and a normal serum magnesium, patients 1, 3, 4, 5, 8, 9 can be classified as Bartter's syndrome. For three patients the classification remains uncertain.
Patients number 2 had a decreased calcium excretion. The glomerular filtration rate of this patient decreased progressively (31 ml/min/173 SA, at the age of 16) and his serum magnesium became lowered since the age of 4 years.
Patient number 6 had a slightly decreased concentrating capacity with a lowered calcium excretion and a decrease of the glomerular filtration rate at the age of 7 years. Occasionally she has a lowered serum magnesium (0.65 mmol/l).
Patient number 7 had a normal concentrating capacity, a lowered calcium excretion and a normal glomerular filtration rate. The serum magnesium level was repeatedly normal.

Table V. Characteristics of Bartter's and Gitelman's syndrome.

	Bartter syndrome's	Gitelman syndrome's
serum magnesium	almost always normal	decreased
urinary excretion of calcium	increased or normal	decreased or normal
concentrating capacity	decreased	normal or slightly decreased
glomerular filtration rate	decreased (or declining) or normal	normal
localisation of defect	thick ascending limb of loop of Henle (medullary part cortical part?)	cortical portion of thick ascending limb and/or distal tubule

It is possible to make a clear distinction between Bartter's syndrome and Gitelman's syndrome. There remain, however, patients with characteristics of both syndromes (table V). The presumed localisation of the defect in the distal nephron is different in both syndromes.

Based on the facts of a disturbance in chloride transport in the distal nephron and the decreased concentrating capacity a defect in the medullary part of the thick ascending limb of the loop of Henle is straight forward. It is difficult to make a definite conclusion about the contribution of the cortical part of the thick ascending limb of the loop of Henle. Each hypothesis is based on micropunction studies in animals. And this part of the nephron behaves differently in the rat and the mouse. In the rat the adenylcyclase system of medullary and cortical portions of the ascending limb are sensitive to ADH, glucagon and calcitonin. Only the cortical part is sensitive to PTH. In the mouse the cortical part is sensitive to all tested hormones. Whereas in the medullary part the adenyl cyclase system was only stimulated by ADH and glucagon. In the control situation in the mouse the calcium and magnesium resorption is almost limited to the cortical part of the ascending limb [14,15]. In the rat the calcium transport is passive in the medullary segments and probably active in the cortical segments [16]. It is thus quite possible that also the cortical part of the ascending limb of Henle is involved in the pathogenesis of Bartter's syndrome.

Based on the fact that the concentrating ability of the patients with Gitelman's syndrome is normal or only slightly decreased together with a disturbance of chloride transport in the distal nephron excludes the medullary part of Henle's loop as localisation of the defect. A moderate concentrating defect can easily be explained by potassium depletion [17]. A defective functioning of the cortical ascending limb or early distal tubule or both can explain the pathogenesis of Gitelman's syndrome.

The combination of hypocalciuria with increased renal magnesium loss was observed by us in patients with isolated renal magnesium loss [18] and in patients after the administration of cisplatin [19].

By applying the Bartoli technique [20] in 5 patients with Gitelman's syndrome, Rodriguez-Soriano et al. indicated the distal tubule as localisation of the defect [21]. By measuring the difference in the urine flow rate after and before furosemide administration during water diuresis, separate calculations of natriumchloride reabsorption by the loop of Henle and by the distal tubule can be performed. The small number of both patients and controls studied do not allow a definite conclusion. Both the free water formed by the loop of Henle and the free water formed by the distal tubule were lowered in Gitelman's syndrome but the difference was only significant for the distal tubule (5 patients versus 4 controls).

In the future the molecular approach will afford the definite solution.

If is confirmed that magnesium suppletion in patients with Gitelman's syndrome, normalises the urinary calcium excretion [22] we have only to explain a combined chloride and magnesium defect, both actively transported in the cortical part of the ascending thick limb of Henle.

Magnesium metabolism

In five out of seven patients with a lowered serum magnesium, the magnesium content of the erythrocytes was also decreased. In experimental studies in the dog the erythrocyte magnesium content behaved as a good indicator for body magnesium stores [23,24]. There is also evidence that mononuclear cell magnesium levels may reflect skeletal and cardiac muscle concentrations [25]. In a similar study in one

patient with Gitelman's syndrome Bianchetti et al. [26] found normal intracellular levels of magnesium in erythrocytes and lymphocytes. In four adult patients with Bartter's syndrome (or Gitelman's syndrome?) Rudin et al. [3] could not demonstrate a reduced magnesium content in the skeletal muscle specimens. All these results do not indicate a pronounced intracellular magnesium deficiency.
An intracellular magnesium deficiency, however, may be more extensive in some exceptional cases such as the patient described by Cushner et al. [27] with a serum magnesium of 0.33 mmol/l. In such a situation supplementation of magnesium might induce a positive potassium balance.
In earlier studies by Rodriguez-Soriano et al. [21] in children with Gitelman's syndrome, a lowered threshold for magnesium could be measured. This was confirmed by our studies in two patients. In addition a normal tubular maximum was present.

ACKNOWLEDGEMENTS

Th. de Boo and W. Lemmens were helpful with the statistical calculations. This study was supported by the Dutch kidney foundation.

REFERENCES

1. Gill JR, Santos F, Chan JC. Disorders of potassium metabolism. In Chan LCM, Gill JR, ed Kidney electrolyte Disorders. Churchill Livingstone, Edinburgh: 1990 137-170.
2. Mace JM, Hambidge KM, Gotlin RW, Dubois RS, Solomons CS, Katz F H Magnesium supplementation in Bartter's syndrome Arch Dis Childh 1973, 78 485-487
3. Rudin A, Bosaeus I, Hessov I. Total body potassium, skeletal muscle potassium and magnesium in patients with Bartter's syndrome. Scand J Clin Lab Invest 1990, 50. 273-277.
4. Fiselier T, Lijnen P, Monnens L, van Munster P, Jansen M, Peer P. Levels of renin, angiotensin I and II, angiotensin converting enzyme and aldosterone in infancy and childhood. Eur J Pediatr 1983, 141: 3-7.
5. Knoers N, Fiselier T, Thomas C, van de Berg R, Theeuwes A, Monnens L. Urinary excretion of prostaglandins during infancy and childhood: influence of age, sodium restriction and posture. Prostaglandins, leucotrienes and essential fatty acids 1990, 39. 295-301.
6. Geven WB, Thomas CMG, Vogels-Mentink GM, Willems JL, Monnens LAH. Renal prostaglandin excretion in patients with isolated hypomagnesemia. Magnesium-Bull 1989, 11. 64-67.
7. Monnens L, Smulders Y, van Lier H, de Boo T. DDAVP test for assessment of renal concentrating capacity in infants and children. Nephron 1981; 29 151-154.
8. Rodriguez-Soriano J, Vallo A, Castillo G, Oliveros R Renal handling of water and sodium in infancy and childhood: a study using clearance methods during hypotonic saline diuresis. Kidney Int 1981; 20. 700-704.
9. Geven W B, Vogles-Mentink G M, Willems J L, de Boo Th, Lemmens W, Monnens L A H Reference values of magnesium and potassium in mononuclear cells and erythrocytes of children. Clin Chem 1990; 36: 1323-1327.
10. Rude RK, Cohan BW, Singer FB. Hypomagnesemia of renal origin: abnormal renal magnesium threshold and normal Tm Mg Magnesium 1983; 2: 62-69.
11. Paunier L, Borgeaud M, Wijss M. Urinary excretion of magnesium and calcium in normal children. Helv Ped Acta 1970, 25· 577-584.
12. Ghazali S, Barratt TM. Urinary excretion of calcium and magnesium in children. Arch Dis Childh 1974; 49: 97-101

13. Gitelman HJ, Graham JB, Welt LG. A familial disorder characterized by hypokalaemia and hypomagnesemia. Ann Ny Acad Sci 1969; 162: 856-864.
14. Quamme GA. Control of magnesium transport in the thick ascending limb. Am J Physiol 1989; 256: F197-F210.
15. De Rouffignac C, Elalouf JM, Roinel N. Histological control of the urinary concentrating mechanism by peptide hormones. Kidney Int 1987; 31: 611-623.
16. Rouse D, Suki WN. Renal control of extracellular calcium. Kidney Int 1990; 38: 700-708.
17. Rosa RM, Epstein FH, Stoff JS. The renal concentrating defect associated with potassium depletion is independent of prostaglandin E2. Am J Kidney Dis 1990; 16: 473-477.
18. Geven W B, Monnens L A, Willems H L, Buijs W C, ter Haar B G. Renal magnesium wasting in two families with autosomal dominant inheritance. Kidney Int 1987; 31: 1140-1144.
19. Bianchetti MG, Kamaka C, Ridolfi-Lüthy A, Wagner HP,, Hirt A, Paunier L, Peheim E, Oetliker OH. Chronic renal magnesium loss, hypercalciuria and mild hypokalaemic metabolic alkalosis after cisplatin. Pediatr Nephrol 1990; 4: 219-222.
20. Bartoli E, Satta A, Faedda R, Olmeo NA, Soggia G, Branca G. A furosemide test in the functional evaluation of the human nephron in vivo. J Clin Pharmacol 1983; 23: 56-64.
21. Rodriguez-Soriano, J, Vallo A. Familial hypokalaemia-hypomagnesaemia. Gitelman's syndrome. Pediatr Nephrol 1990; 4: C22.
22. Rodriguez-Soriano J, Vallo A, Garcia-Fuentes, M. Hypomagnesemia of hereditary renal origin. Pediatr Nephrol 1987; 1: 465-472.
23. Geven W, Vogels-Mentink G, Willems J, de Boo Th, Lemmens W, Monnens L. Experimental magnesium depletion in the dog. Influence of the magnesium content of mononuclear cells, erythrocytes, muscle and bone. Mag Bull 1988; 10: 45-50.
24. Geven W, Vogels Mentink G, Willems J, Joordens J, Hilbers C, Monnens L. Experimental magnesium depletion in the dog. Relation between total and ultrafiltrated Mg in plasma and total and ionized Mg in erythrocytes. Mag Bull 1990; 12: 166-169.
25. Elin RJ. Status of the determination of magnesium in mononuclear blood cells in humans. Magnesium 1980; 7: 300;305.
26. Bianchetti MG, Girardin E, Benador-Milsztajn N, Sinozenko PC, Paunie L. Metabolic studies in primary tubular hypomagneseamia-hypokalaemia. Mag Res 1988; 1: 79-83.
27. Cushner HH, Peller TP, Fried T, Delea CS. Does magnesium play a role in the hypokalemia of Bartter's syndrome. Am J Kidney Dis 1990; 16: 495-500.

Chapter 10

SUMMARY AND FINAL DISCUSSION

Magnesium deficiency has many different causes. The underlying pathophysiology is based on three different types of defects: lowered intake, disturbed intestinal resorption or decreased renal reabsorption [1].
The kidney is considered to be the key organ in the magnesium homeostasis [2]. After ultrafiltration in the glomerulus about 25% of the filtrated magnesium is reabsorbed in the proximal tubule and up to 65% is reabsorbed in the thick ascending limb of Henle, depending on the level of magnesium in the blood. In contrast with the total number of patients with renal magnesium loss, patients showing congenital impairment of renal magnesium reabsorption are rare [3]. Three different types of hereditary renal magnesium loss are distinguished by Rodriguez-Sariano [4]: isolated familial hypomagnesemia, familial hypokalemia-hypomagnesemia and familial hypomagnesemia-hypercalciuria (chapter 1).

Isolated renal magnesium loss

In chapter 2, two unrelated families with isolated renal magnesium loss are described showing an autosomal dominant mode of inheritance. No abnormalities were detected by conventional kidney function tests performed in both probands and by kidney biopsy in one. Magnesium infusion tests showed not only a lowered renal magnesium threshold but also a reduced tubular maximum for magnesium. With oral magnesium supplementation, we were unable to correct the lowered serum magnesium level.
It is important to realise that renal magnesium reabsorption is not characterised by a tubular transport capacity as may be supposed at inspection of the data of the urinary excretion. In fact, micropuncture data during magnesium loading in the dog and rat reveal that in the proximal tuble net reabsorption of magnesium rose proportional to the increase in filtered load and increased initially in Henle's loop but fell with high plasma magnesium concentrations [5,6]. Little magnesium reabsorption was observed between distal collection site and final urine.
When a normal level of ultrafiltrable magnesium was reached during the magnesium loading test in patients with renal magnesium loss, about half of the filtered magnesium was excreted in the urine. This indicates the ascending limb of Henle's loop as at least one of the localisations of disturbed magnesium transport.
In all members of both families with hypomagnesemia, a lowered urinary excretion of calcium was noticed. As is discussed in chapter 9 about Bartter/Gitelman's syndrome, the cortical part of the ascending limb of Henle's loop should be involved.
Isolated renal magnesium loss with autosomal recessive inheritance is described in two sisters in chapter 3. Magnesium infusion test in both girls revealed a lowered magnesium threshold and a normal tubular maximum. It is difficult to understand what is altered in the magnesium transport when only the threshold is lowered. In opposite to the autosomal dominant isolated renal magnesium loss, calcium excretion is normal in the autosomal recessive form.

Prostaglandin excretion during hypomagnesemia

Prostaglandin studies during magnesium depletion, meanly performed in rats [7,8], showed elevated plasma and tissue prostaglandin levels. In chapter 4 renal prostaglandin excretion in patients with isolated renal hypomagnesemia is presented.

Urinary excretion of prostaglandin E_2, prostaglandin $F_{2\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$ and tromboxane B_2 was in the normal range. Species differences, and different degree and duration of magnesium deficiency may exist. In contrast to potassium depletion, hypomagnesemia in humans does not stimulate the excretion of prostaglandins in the urine.

INTRACELLULAR MAGNESIUM CONTENT

Reference values in children

In chapter 5 the reference values of serum magnesium concentration, as well as magnesium and potassium content in mononuclear cells and erythrocytes are presented. Samples were taken from cord blood and in children from infancy through adolescence. Differences between boys and girls were absent. Mononuclear magnesium content was independent of age and was within the adult range of values. No significant correlation between the serum magnesium level and the mononuclear magnesium content was shown. Mononuclear potassium also showed no age-related differences. A significant correlation between mononuclear magnesium and potassium content was observed; however this correlation was lower when magnesium and potassium contents were expressed in terms of protein content.
The magnesium content of erythrocytes was significantly lower in cord blood and during the first month of live, compared with that at older ages and showed no significant correlation with the serum magnesium level. The lower magnesium content in the erythrocyte expressed per gram dry weight is related to the decreased number of erythrocytes per gram dry weight and thus a decreased cell membrane content per gram dry weight. About 6% of the erythrocyte magnesium content might be bound to the cell membranes [9].
The erythrocyte potassium concentration was independent of age and a low but significant correlation with erythrocyte magnesium content was noted.

Intracellular magnesium content in patients with isolated renal magnesium loss

In spite of remarkably lowered serum magnesium levels, mononuclear magnesium content was in the normal range in patients with isolated renal magnesium loss (chapter 4). Erythrocyte content of magnesium was lowered in 6 out of 12 patients and was in the low normal range in 4 others. A significant correlation between erythrocyte magnesium content and the serum magnesium level was noted in these patients.

Studies in patients with Barrter/Gitelman's syndrome

In chapter 9, patients with Bartter's syndrome were distinguished from patients with Gitelman's syndrome based on the serum magnesium level, the urinary excretion of calcium, the concentrating ability and the glomerular filtration rate. In five of seven patients with a lowered serum magnesium level, a reduced magnesium content in the erythrocytes was observed. The erythrocyte magnesium content is considered to be a

good reflection of the body magnesium stores.

Experimental magnesium depletion

Experimental magnesium deficiency was induced in young adult beagles by magnesium deficient diet as described in chapter 6. A significant decrease of the magnesium content of muscle and mononuclear cells was not detected during and at the end of the magnesium deficiency, but the muscular magnesium content rose significantly after normalisation of the serum magnesium level.
The serum magnesium concentration and both erythrocyte and bone magnesium content decreased significantly during and at the end of the magnesium deficiency period. A highly positive correlation coefficient for serum and erythrocyte magnesium was present and positive correlation was shown for erythrocyte and bone magnesium amounts. The wide range of the bone magnesium levels before the start of the magnesium deficient diet, indicates that individual measurements of the bone magnesium content have limited applicability for the detection of intracellular magnesium deficiency. There was a low correlation coefficient between serum magnesium and bone magnesium during magnesium depletion in the dog.
This finding is in contrast with the data presented by Cohen and Kitzes [10] in humans; they found a very good correlation between the serum magnesium concentration and bone magnesium content.
Erythrocyte magnesium content and serum magnesium showed a good correlation. After normalisation of the serum magnesium concentration the erythrocyte magnesium content rose significantly compared with the values at the end of the magnesium deficient period. The results of these experimental studies agree with those obtained in patients with renal magnesium loss. Erythrocyte magnesium levels are a better reflection of the body magnesium stores than mononuclear cells. This important place of the erythrocyte stimulated further studies of the metabolic active component of magnesium in the erythrocytes. After a short review of the literature, comment on the own obtained results will be made.

MAGNESIUM TRANSPORT IN ERYTHROCYTES

Magnesium influx

Examinations of ^{28}Mg uptake by red cells in several species revealed a very slow uptake in human erythrocytes but about ten times faster in rat, cat and dog red cells [11]. In human erythrocytes about 5% of the plasma ^{28}Mg amount was exchanged for intra-erythrocyte magnesium in 5 hours [11]. Adsorption of magnesium to the external membrane of the erythrocyte should, however, be excluded. No uptake of ^{28}Mg by human erythrocytes incubated 24 hours at 37° C was detected by Ginsburg et al. [12]. The explanation of this phenomenon is that the magnesium ion binds strongly 6 molecules of water and exchanges this water very slowly [13] for polar groups in the channel wall in the membrane [14]. Since dehydration at a narrow region of the channel is the rate-limiting step in the passage of ions through the membrane, the low permeability of membranes for magnesium is not surprising [14]. It is suggested that

magnesium movements occurring in red cells will be in association with membrane protein [15].
Ginsburg et al. showed that magnesium content of human and rabbit erythrocytes in suspension increased as the reticulocyte count increased [12]. Recently in guinea pig reticulocytes and mature red cells Jelics et al. [16] detected a sharply decreased ATP concentration in mature red cells compared to reticulocytes, whereas the intracellular free magnesium concentration increased from 150 μmol/l cell water in reticulocytes to 250 μmol/l cell water in mature red cells [16]. It was suggested that alterations in the kinetics of membrane ion transport systems, accompanying changes in cholesterol and phospholipid content, occur during maturation of the red cell [16]. In contrast to the lowered level of free intracellular magnesium, the total magnesium content is increased in the reticulocytes. A large amount of magnesium is bound to ATP and magnesium may also be bound to the ribosomes.

Magnesium efflux

The initial rate of magnesium efflux in fresh human erythrocytes incubated in Na^+, K^+-Ringer's medium was 7.3 ± 2.8 μmol/l cells per hour (mean ± sd) [17]. Lüdi and Schatzman [18] detected a Na^+-stimulated maximal magnesium efflux of 4.6 ± 1.2 μmol/l cells per hour (mean ± sem) in unloaded human erythrocytes. The normal content of total magnesium in the erythrocyte is about 3 mmol/l cell water. This means the about 0.2 per cent of the magnesium content is leaving the red blood cell per hour.
In the last few years several papers characterising net magnesium efflux from magnesium loaded red cells were published [19-27]. In the magnesium loaded cells, however, the fluxes are much larger than in magnesium unloaded erythrocytes. A Na^+-dependent and a Na^+-independent magnesium efflux was demonstrated [21].
Na^+-dependent magnesium efflux in rat and chicken erythrocytes was remarkably higher than Na^+-independent efflux (5.7 and 4.2 times respectively) [24]. This is in contrast to loaded human red cells in which Na^+-dependent magnesium efflux was 0.16 mmol/l cells per 30 min whereas Na^+-independent magnesium efflux amounted 0.89 mmol/l cells per 30 min. [24]. The Na^+-dependent magnesium efflux (in rat erythrocytes) only occurred at elevated intracellular magnesium amounts and stopped when the physiological magnesium content was reached. Magnesium efflux was specifically combined with the uptake of sodium at a stoichiometric ratio of $2Na^+$: $1Mg^{2+}$, indicating electroneutral Na^+/Mg^{2+} antiport. This Na^+/Mg^{2+} antiport (in human and rat erythrocytes) is ATP dependent[19,27].
Na^+-independent magnesium efflux in loaded human erythrocytes (about 90% of total net magnesium efflux) was considered to be dependent on net chloride efflux for charge compensation [23]. Both effluxes are inhibited by amiloride [25].
In magnesium loaded erythrocytes from magnesium deficient rats both Na^+-dependent and Na^+-independent magnesium efflux were increased by one third and it was suggested that increased affinity of the Na^+/Mg^{2+} antiporter for intracellular magnesium caused the reduced intracellular magnesium concentration [26]. This efflux system is unaltered during maturation of erythrocytes [27].

Ionized magnesium in erythrocytes; comment on the own obtained results

Ionized magnesium concentration of human erythrocytes ($[Mg^{2+}]_i$) is 0.55 ± 0.12 mmol/l cell water (chapter 8) measured with zeropoint titration. ^{31}P Nuclear Magnetic Resonance (^{31}P NMR) measurements of $[Mg^{2+}]_i$ revealed 0.20 ± 0.03 mmol/l cell water. Variation in the dissociation constants markedly influences the results of intracellular magnesium when determined by the ^{31}P NMR method. Lowered $[Mg^{2+}]_i$ was detected with ^{31}P NMR in magnesium deficient dogs (chapter 7) and with both methods in patients with isolated renal magnesium loss (chapter 8).

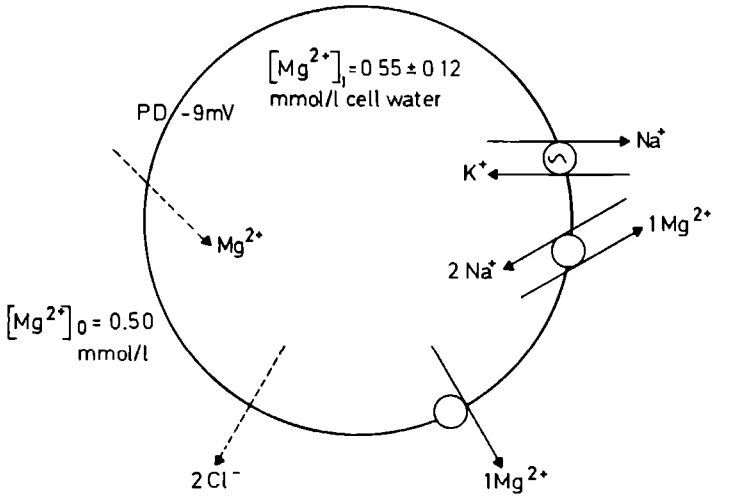

Figure 1. Tentative model of magnesium transport systems in the human erythrocyte. Passive transport is denoted by dashed arrows, carrier-mediated transport by solid arrows and active transport processes are indicated by the ~ symbol.

Erythrocyte $[Mg^{2+}]_i$ is below the electrochemical equilibrium

In controls in which ultrafiltrable magnesium in plasma is 0.57 ± 0.04 mmol/l corresponding with an ionized magnesium concentration of ≈ 0.5 mmol/l, a $[Mg^{2+}]_i$ of 0.5 mmol/l cell water is below the electrochemical equilibrium. With a membrane potential about 9 mV, inside (negative) the expected $[Mg^{2+}]_i$ should be ≈ 1 mmol/l cell water in a situation where electrochemical equilibrium exists. In magnesium deficient rats [27] and dogs (chapter 7) ionized plasma magnesium is lowered just like $[Mg^{2+}]_i$ and it is clear that in this situation, $[Mg^{2+}]_i$ is also below the electrochemical equilibrium. Also in patients with isolated renal magnesium loss in which both plasma ionized magnesium and $[Mg^{2+}]_i$ are lowered, $[Mg^{2+}]_i$ = 0.36 mM while $[Mg^{2+}]_i$ would be 0.59 mmol/l cell water in the situation of electrochemical equilibrium (chapter 8).

SUGGESTIONS FOR FUTURE INVESTIGATIONS

Perinatology

Serum magnesium was significantly lower in cord blood than in samples from age groups older than one month. The concentration of magnesium in erythrocytes was also significantly lower in cord blood and during the first month of life compared to samples obtained in older children. Fetal plasma magnesium, however, exceeds that of the mother. Further studies are needed to measure not only the total magnesium concentration in serum but also the ultrafiltrable magnesium concentration as an indicator of ionized magnesium. The level of ionized magnesium in serum should be related to the parathormone level.
The compartmentalisation of intracellular magnesium in the erythrocytes needs evaluation. What is the role of fetal haemoglobin?

Erythrocytes

Although still matter of debate, increasing evidence is available that magnesium exchange exists in erythrocytes under physiological conditions. In the magnesium deficient state in animals (rats and dogs) and humans [28], decreased values for ultrafiltrable magnesium in plasma and ionized and total magnesium in erythrocytes were observed. As presented in chapter 7 total erythrocyte magnesium decreased at a significant slower rate than ultrafiltrable plasma magnesium. The hypothesis that the quick fall of ultrafiltrable plasma magnesium (c.q. ionized magnesium) induces relative fast magnesium efflux out of the erythrocyte leading primarily to lowering of $[Mg^{2+}]_i$ followed by a drop of the total magnesium content of erythrocytes, needs experimental confirmation.

Intracellular magnesium

As fluorescent probes become available for the measurement of cytosolic free magnesium concentration, this method should be applied and compared with classical techniques.
After the introduction of a new family of fluorescent calcium chelators by Grynkiewicz et al. [29], fluorinated NMR active derivates of the chelator 0-aminophenol-N,N,O-triacetic acid (APTRA) for the measurement of cytosolic free magnesium concentration were synthethised by Levy et al. [30]. The lipofylic acetomethylester of APTRA easily enters cells, including red blood cells, where intracellular cytosolic esterases cleave the ester, leaving the negatively hydrofylic charged, free acid trapped in the cell. ^{31}F NMR studies of 4-methyl-5-fluoro-APTRA-loaded human erythrocytes revealed a basal ionized magnesium level of 0.25 mM. In cultured chick heart cells $[Mg^{2+}]_i$ was 0.56 ± 0.03 mM. [31]. APTRA is insensitive to pH variations near the normal physiological range and ^{31}F NMR studies revealed that both magnesium and calcium ions are bound by APTRA derivates. However significant differences are present in the APTRA-magnesium and APTRA-calcium dissociation constants. For example for 4-methyl-5-fluoro APTRA the Kd^{Mg} = 0.9 ± 0.1 mM and the Kd^{Ca} = 25 ± 10 mM at 37° C. [30]. Moreover cytosolic calcium is present at a too low level to

significantly complex the chelator.
Recently Raju et al. [32] modified APTRA to yield a fluorescent analogue which can be utilised as an intracellular probe for ionized magnesium. The chelator is called FURAPTRA or Mag-FURA-2 and has the desired sensitivity to cytosolic magnesium (Kd^{Mg} = 1.5 mM) and is insensitive to pH and ionized calcium levels in the physiological range. After transport into the cell, the ester of FURAPTRA is hydrolysed and the free acid is than complexed with cytosolic ionized magnesium, resulting in excitation shift of this fluorescent indicator. The fluorescence changes of the absorption spectrum after magnesium complexation are similar to those observed on complexation of FURAPTRA by calcium ions, enabling the 'dual excitation ratio' method [29]. The advantage of this method is that measurement is independent of the absolute intracellular FURAPTRA concentration and that the calibration procedure is easier in comparison to a 'single wavelength' method. Two more advantages of FURAPTRA over the fluorinated NMR active derivates of APTRA are that FURAPTRA can provide a better time resolution and is applicable when the total number of cells available for study is limited. In this way $[Mg^{2+}]_i$ can be measured with a frequency of 5 per second by a photon-counting spectrofluorometer using, for instance, videoimaging. Incomplete hydrolysis of the acetoxymethyl ester of Indo-1 in epithelial cells [33], and incomplete deesterification of the acetoxymethyl ester of Fura-2 in human polymorphonuclear leukocytes [34], is reported. The same might apply to FURAPTRA. Partial hydrolysis products that fluoresce but do not bind with the same affinity as the free acid of Mag-FURA-2, can cause errors in the measurements of ionized magnesium.
Several investigations of $[Mg^{2+}]_i$, where FURAPTRA was used, were reported recently [35-37].
In isolated rat hepatocytes cytosolic $[Mg^{2+}]_i$ was 0.59 mM [32].
In cultured chicken heart cells basal cytosolic ionized magnesium averaged 0.48 ± 0.03 mM. Perfusion with sodium-free solution increased $[Mg^{2+}]_i$ threefold [36]. The increase of $[Mg^{2+}]_i$ largely attenuated when calcium was removed from the sodium-free perfusate, so it was concluded that a substantial part of the increase of $[Mg^{2+}]_i$ was dependent on an increase of intracellular ionized calcium. It was suggested that $[Mg^{2+}]_i$ is altered by calcium, most likely due to competition for intracellular binding sites [35].
In isolated beef heart mitochondria matrix, $[Mg^{2+}]_i$ was near 0.50 mM in the absence of external magnesium [36]. Matrix $[Mg^{2+}]_i$ increased when external magnesium increased. Matrix free $[Mg^{2+}]_i$ changed with changing ligand availability and such changes contribute to the regulation of $[Mg^{2+}]_i$-sensitive enzymes and transporters [36]. Addition of Pi decreases the free magnesium concentration.
Intracellular free magnesium in cortical thick ascending limb cells is 0.53 ± 0.05 mM. About 2% of the total cell magnesium content should be free ionized magnesium [37]. Accordingly Mg^{2+} can enter the cell down the electrochemical gradient for magnesium is actively removed out of the cell.
In culture techniques the magnesium concentration of the perfusate may alter $[Mg^{2+}]_i$, so they are less suitable for research of magnesium deficiency in animals and humans. Direct measurement of $[Mg^{2+}]_i$ in erythrocytes or leucocytes with FURAPTRA may

provide a further step forward in magnesium research in the normal and magnesium deficient state.

Study of the requirements of a correct measurement in these cells is necessary before they can be introduced into clinical medicine. It is an unique opportunity for a combination of basic and clinical research.

REFERENCES

1. Thorén, L. Magnesium metabolism. Progr. Surg. 1971; 9: 131-156.
2. Quamme, G.A. Control of magnesium transport in the thick ascending limb. Am. J. Physiol. 1989; 256: F197-F210.
3. Labeeuw, M., Pozet, N. Magnesium in kidney diseases. A review. Mag. Res. 1988, 1: 187-202.
4. Rodriguez-Soriano, J., Vallo, A., Garcia-Fuentes, M. Hypomagnesaemia of hereditary renal origin. Pediatr. Nephrol. 1987; 1: 465-472.
5. Wong, N L.M., Dirks, J.H., Quamme, G.A. Tubular reabsorptive capacity for magnesium in the dog kidney. Am. J. Physiol. 1983, 244: F78-F83.
6. Quamme, G.A., Dirks, J.H. Intraluminal and contraluminal magnesium on magnesium and calcium transfer in the rat nephron. Am J. Physiol. 1980. 238: F187-F198
7. Nigam, S., Averdonk, R , Günther, T. Alteration of prostanoid metabolism in rats with magnesium deficiency. Prostagl. Leuk. Med. 1986, 23: 257-263
8. Soma, M., Cunnane, S.C., Horrobin, D.F., Manku, M.S., Honda, M , Hatono, M Effects of low magnesium diet on the vascular prostaglandin and fatty acid metabolism in rats. Prostaglandins. 1988; 36: 431-441.
9. Fujii, T., Sato, T., Hanzawa, T. Calcium and magnesium contents of mammalian erythrocyte membranes. Chem. Pharm. Bull 1973, 21. 171-175.
10. Cohen, L., Kitzes, R Relationship of bone and plasma magnesium in magnesium-deficient cirrhosis patients. Isr. J. Med. Sci. 1982; 18: 679-682.
11. Rogers, T.A. The exchange of radionactive magnesium in erythrocytes of several species J Cell Comp. Phys 1961; 57: 119-121.
12. Ginsburg, S., Smith, J.G., Ginsburg, F M., Reardon, J Z., Aikawa, J.K. Magnesium metabolism of human and rabbit erythrocytes. Blood. 1962, 20: 722-729.
13. Hille, B. Ionic channels of excitable membranes Sinauer Associates Inc Sunderland Mass 168-171.
14. White, R.E., Hartzell, H.C. Magnesium ions in cardiac function. Biochem. Pharmacol. 1989, 38: 859-867.
15. Flatman, P.W. The control of red cell magnesium. Mag. Res. 1988, 1. 5-11.
16. Jelicks, L.A., Weaver, J., Pollack, S, Gupta, R.J. NMR studies of intracellular free calcium, free magnesium and sodium in the guinea pig reticulocyte and mature red cell Biochem Biophys. Acta. 1989; 1012: 261-266.
17. Féray, J-C., Garay, R. A one-to-one Mg^{2+}:Mn^{2+} exchange in rat erythrocytes J Biol Chem 1987; 262: 5763-5768.
18. Lüdi, H., Schatzmann, H.J. Some properties of a system for sodium-dependent outward movement of magnesium from metabolising human red blood cells. J Physiol. 1987, 390. 367-382.
19. Frenkel, E.J., Graziani, M., Schatzmann, H.J. ATP requirement of the sodium-dependents magnesium extrusion from human red blood cells. J. Physiol 1989, 414. 385-397
20. Féray, J-C., Garay, R. An Na^+-stimulated Mg^{2+}-transport system in human red blood cells. Biochem. Biophys. Acta. 1986; 856: 76-84.
21. Günther, T., Vormann, J., Förster, R. Regulation of intracellular magnesium by Mg^{2+} efflux. Biochem. Biophys. Res. Commun. 1984; 119: 124-131
22. Günther, T., Vormann, J. Mg^{2+} efflux is accomplished by an amiloride-sensitive Na^+/Mg^{2+} antiport. Biochem Biophys. Res. Commun. 1985; 130: 540-545

23. Gunther, T., Vormann, J Na^+-independent Mg^{2+} efflux from Mg^{2+}-loaded human erythrocytes. FEBS Lett. 1989, 247: 181-184
24. Gunther, T., Vormann, J. Characterization of Mg^{2+} efflux from human, rat and chicken erythrocytes FEBS Lett. 1989, 250: 633-637.
25. Günther, T, Vormann, J., Gragoe Jr, E.J., Höllriegl, V. Characterization of Na^+-dependent and Na^+-independent Mg^{2+} efflux from erythrocytes by amiloride derivates Mag Bull. 1989, 11: 103-107.
26. Gunther, T., Vormann, J, Höllriegl, V. Concentration of intracellular free Mg^{2+} and Mg^{2+} efflux from magnesium- deficient erythrocytes. Mag.-Bull. 1990, 12 43-47.
27. Gunther, T., Vormann, J. Characterization of Na^+-independent Mg^{2+} efflux from erythrocytes. FEBS Lett. 1990; 271 149-151.
28. Ryzen, E., Servis, K.L., DeRusso, P., Kershaw, A., Stephan, T., Rude, R K. Determination of intracellular free magnesium by Nuclear Magnetic Resonance in human magnesium deficiency. J. Am. Coll. Nutr. 1989; 8: 580-587.
29. Grynkiewicz, G, Poenie, M., Tsien, R.Y. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties J. Biol. Chem. 1985, 260. 3440-3450
30. Levy, L A., Murphy, E., Raju, B., London, R E Measurement of cytosolic free magnesium ion concentration by ^{19}F NMR Biochem. 1988, 27 4041-4048
31. Rotevatn, S, Murphy, E., Levy, L.A., Raju, B., Lieberman, M., London, R E. Cytosolic free magnesium concentration in cultured chick heart cells Am. J. Physiol. 1989; 257: C141-C146.
32. Raju, B, Murphy, E., Levy, L.A., Hall, R.D., London, R E. Afluorescent indicator for measuring cytosolic free magnesium Am. J. Physiol 1989, 256 C540-C548
33. Lückhoff, A. Measuring cytosolic free calcium concentration in endothelial cells with indo-1. the pitfall of using the ratio of two fluorescence intensities recorded at different wavelength. Cell Calcium. 1986; 7: 233-248
34. Scallon, M., Williams, D A., Fay, F S A Ca^{2+}-insensitive form of Fura-2 associated with polymorphonuclear leukocytes. Biol Chem 1987; 262 6308-6312.
35. Murphy, E, Freudenrich, G C., Levy, L.A., London, R.E. Monitoring cytoslic free magnesium in cultured chicken heart cells by use of the fluorescent indicator Furaptra Proc Natl. Acad Sci. USA. 1989, 86 2981-2984.
36. Jung, D.W., Apel, L, Brierley, G P. Matrix free Mg^{2+} changes with metabolic state in isolated heart mitochondria. Biochem. 1990, 29: 4121-4128.
37. Quamme, G A., Dai, L-J. Control of cytosolic free Mg^{2+} in cortical thick ascending limb cells. J. Am. Soc Nephrol. 1990, 1: 581.

SAMENVATTING

Hoewel Magnesium reeds door de mens in de middeleeuwen werd gebruikt als laxeermiddel, drong pas in de 20er en 30er jaren van deze eeuw het besef door dat magnesium een essentieel element is voor mens, dier en plant. Hoewel aanvankelijk getwijfeld werd aan het bestaan van een magnesium deficiëntie syndroom werd dit in 1934 voor het eerst door Hirsfelder bij de mens beschreven.
In hoofdstuk 1 worden naast de verschijnselen die zijn beschreven bij magnesium tekort, de ziekten en afwijkingen beschreven die (kunnen) lijden tot magnesium deficiëntie. De onderliggende pathofysiologie is gebaseerd op drie soorten afwijkingen: verminderde opname, gestoorde intestinale resorptie of verminderde renale reabsorptie.
Het handhaven van een normale magnesium spiegel in het serum vindt plaats in de nier. Na glomerulaire filtratie wordt in de proximale tubulus ongeveer 25% teruggeresorbeerd en ± 65% in het opstijgende been van de lis van Henle. Afhankelijk van een (te) lage of (te) hoge serum Mg spiegel wordt in dit segment van de nier magnesium nagenoeg volledig gereabsorbeerd of slechts voor een (klein) gedeelte. Mg reabsorptie kan zowel transcellulair als paracellulair plaatsvinden.
Geïsoleerd renaal magnesium verlies met autosomaal dominante overerving in twee families wordt in hoofdstuk 2 beschreven. Het is opvallend dat de personen die een sterk verlaagde serum Mg spiegel hebben geen verschijnselen hebben van magnesium deficiëntie. De opname van Mg in de darm is ongestoord. Met standaard nierfunctie onderzoek in beide probandi en in een nierbiopsie in een van beide, werden geen afwijkingen vastgesteld. Magnesium infusie studies tonen een verlaagde nierdrempel voor Mg aan en tevens een verlaagd tubulair maximum. Orale magnesium suppletie resulteerde niet in normaliseren van de serum magnesium spiegel.
Men bedenke dat de renale magnesium reabsorptie niet adequaat kan worden uitgedrukt in termen van tubulaire transport capaciteit. Micropunctie studies bij de hond en de rat hebben aangetoond dat de netto reabsorptie in de proximale tubulus proportioneel toenam met de gefilterde hoeveelheid. In het opstijgende been van de lis van Henle daarentegen neemt de reabsorptie aanvankelijk toe bij stijging van de hoeveelheid gefilterd magnesium, maar neemt af als de serum magnesium spiegel verder stijgt. Slechts geringe magnesium reabsorptie werd vastgesteld in de distale tubulus en de verzamelbuizen.
Als tijdens de magnesium infusie test bij de patiënten een normale ultrafiltrabele Mg spiegel in serum wordt bereikt, wordt ongeveer de helft van het gefilterde magnesium uitgescheiden in de urine. Dit betekent dat in ieder geval het magnesium transport in het opstijgende been van de lis van Henle is gestoord.
Alle personen met hypomagnesiemie hadden een verlaagde calcium uitscheiding in de urine. Zoals ook in hoofdstuk 9 over het Bartter/Gitelman syndroom wordt besproken, ligt het voor de hand dat het corticale deel van het opstijgende been van de lis van Henle hierin is betrokken.
Een familie met autosomaal recessief overervend renaal magnesium verlies wordt beschreven in hoofdstuk 3. Magnesium infusie studies tonen een verlaagde nierdrempel voor magnesium aan met een normaal renaal tubulair maximum.

De stoornis die ten grondslag ligt aan een uitsluitend verlaagde nierdrempel voor magnesium is niet goed te begrijpen In tegenstelling tot de autosomaal dominante vorm van renaal magnesium verlies, wordt bij de recessieve vorm een normale calcium uitscheiding in de urine waargenomen.
Uit dierexperimenteel onderzoek blijkt dat tijdens magnesium deficientie een verhoogd gehalte aan prostaglandines in plasma en weefsels wordt gevonden. Bij personen met geisoleerd renaal magnesium verlies werd daarentegen een normale excretie van $PGF_{2\alpha}$, PGE_2, Tromboxane en 6-keto-$PGF_{1\alpha}$ in de urine vastgesteld (hoofdstuk 4). Verschillen tussen species en een verschil in de duur en/of ernst van de magnesium deficientie zouden hebben kunnen bestaan. In tegenstelling tot kalium depletie, leidt hypomagnesiemie bij de mens niet tot een verhoogde uitscheiding van prostaglandines in de urine.
De referentie waarden voor Mg in serum, mononucleaire cellen en erytrocyten worden beschreven in hoofdstuk 5. Het gehalte van magnesium in mononucleaire cellen is niet verschillend in navelstrengbloed, bij zuigelingen en bij kinderen tot 16 jaar en ligt binnen de volwassen grenswaarden Er werd geen correlatie tussen de serum magnesium spiegel en het magnesium gehalte in mononucleaire cellen waargenomen. Het kalium gehalte in mononucleaire cellen vertoonde geen verschillen met de leeftijd. Er bestond een significante correlatie tussen het magnesium en kalium gehalte in mononucleaire cellen, maar deze correlatie was lager als de magnesium en kalium gehaltes werden uitgedrukt op basis van het eiwit gehalte.
Het serum Mg gehalte in navelstrengbloed is gedurende de eerst levensmaand lager dan daarna
In de erytrocyten in navelstrengbloed en gedurende de eerste maand postpartum is het magnesium gehalte lager dan bij de volwassene, daarna tussen de leeftijd van 1 en 6 maanden wordt een sterke stijging gezien tot volwassen waardes.
De kalium concentratie in erytrocyten is leeftijds on-afhankelijk en vertoont een lage maar significante correlatie met het magnesium gehalte.
Ondanks de duidelijk verlaagde serum magnesium waarden bij de patienten met geisoleerd renaal magnesium verlies, ligt het mononucleair magnesium gehalte bij hen binnen de normaal waarden (hoofdstuk 4). In de erytrocyten is het magnesium gehalte verlaagd bij 6 van 12 patienten en is laag normaal bij 4 anderen. Er werd een significante correlatie tussen magnesium gehaltes in serum en rode bloed cellen vastgesteld
In hoofdstuk 9 wordt een poging ondernomen patienten met het Bartter syndroom te onderscheiden van hen met het Gitelman syndroom, op grond van hun serum magnesium spiegel, de uitscheiding van calcium in de urine, het concentrerend vermogen van de nier en de glomerulaire filtratie snelheid In vijf van zeven patienten met een verlaagde serum magnesium concentratie wordt een verlaagd magnesium gehalte in de erytrocyten waargenomen. Het erytrocytaire magnesium gehalte wordt beschouwd als een goede weerspiegeling de totale lichaams magnesium voorraad
Uit dierexperimenteel onderzoek bij de hond, beschreven in hoofdstuk 6, komt naar voren dat tijdens magnesium deficientie geen daling van het mononucleair- en spier magnesium wordt waargenomen, ondanks langdurig sterk verlaagde serum magnesium concentraties. Vijf van de tien honden vertoonden een betrekkelijk laag bot

magnesium gehalte voorafgaand aan de proef ondanks normale serum spiegels. Bij deze honden werd nauwelijks of geen daling van het bot magnesium vastgesteld tijdens de magnesium deficiëntie periode. Toch waren de bot- en erytrocyten magnesium gehaltes positief gecorreleerd. Individuele meetingen van het magnesium gehalte in bot hebben een beperkte toepasbaarheid voor de detectie van magnesium deficiëntie.
Het magnesium gehalte in rode bloedcellen en de serum magnesium concentratie zijn sterk gecorreleerd. Na herstel van de serum magnesium spiegel stijgt het erytrocytaire magnesium gehalte in vergelijking tot het einde van de magnesium depletie periode. Deze waarnemingen komen overeen met hetgeen werd waargenomen bij patiënten met renaal magnesium verlies. Geconcludeerd werd dat het erythrocytaire magnesium-gehalte een betere parameter is voor het aantonen van magnesium deficiëntie dan dat in mononucleaire cellen. De belangrijke plaats die de erytrocyten innemen leidden tot nader onderzoek naar de metabool actieve vorm van magnesium gehalte in de rode bloed cellen; het geïoniseerde magnesium.
In hoofdstuk 8 worden ^{31}P Nuclear Magnetic Resonanace (31P NMR) en nulpunt titratie als methoden voor het meten van vrij geïoniseerd intracelluliar magnesium beschreven bij 14 gezonde controle personen en 7 personen met erfelijk renaal magnesium verlies. De intracellulaire geïoniseerde magnesium concentratie in humane erytrocyten bedraagt 0.55 ± 0.12 mmol/l cel water, gemeten met nulpunt titratie. ^{31}P NMR metingen van $[Mg^{2+}]_i$ leverden 0.20 ± 0.03 mmol/l cel water op. Variatie in de verschillende dissociatie constanten beïnvloedden het resultaat van de $[Mg^{2+}]_i$ meting met ^{31}P NMR sterk. Een verlaagde $[Mg^{2+}]_i$ werd vastgesteld met ^{31}P NMR in magnesium deficiëntie honden (hoofdstuk 7) en met beide methoden bij patiënten met geïsoleerd renaal magnesium verlies (hoofdstuk 8).
De ultrafiltrabele magnesium concentratie in plasma bedraagt 0.57 ± 0.04 mmol/l bij controle personen; dit komt overeen met een geïoniseerd magnesium concentratie van ongeveer 0.50 mmol/l, terwijl $[Mg^{2+}]_i$ in erytrocyten 0.50 mmol/l cel water is. Dit is beduidend lager dan de concentratie die er zou bestaan in geval van electrochemisch evenwicht. Met een membraan potentiaal die -9 mV binnen in de erytrocyt is, bedraagt de met behulp van de wet van Nernst berekende $[Mg^{2+}]_i$ ± 1 mmol/l cel water indien er electrochemisch evenwicht zou bestaan. Bij patiënten met geïsoleerd renaal magnesium verlies bij wie een verlaagde concentratie van geïoniseerd magnesium in het plasma bestaat en $[Mg^{2+}]_i$ eveneens verlaagd is (0.36 mmol/l cel water), terwijl $[Mg^{2+}]_i$ 0.59 mmol/l cel water zou moeten bedragen indien er sprake zou zijn van electrochemisch evenwicht.
Goede verwachtingen worden uitgesproken van het aanwenden van FURAPTRA voor het meten van de cytoplasmatische geïoniseerde magnesium concentratie.

DANKWOORD

Een proefschrift kan alleen tot stand komen met behulp van een niet onaanzienlijk aantal personen; zo ook in het onderhavige geval.
Bijzondere dank gaat uit naar de patiënten en hun familieleden. Door hun bereidheid en belangeloze medewerking aan grote delen van dit onderzoek kon een wezenlijke verbreding in de kennis van het magnesium metabolisme worden verkregen.
Prof. L.A.H. Monnens; beste Leo, door je unieke manier van begeleiden van het onderzoek maar ook je visie op wetenschap en patiëntenzorg werden de afgelopen jaren niet alleen leerzaam maar ook heel boeiend. Met dit proefschrift is een klein deel van de wens die je vele jaren geleden in de status van een patiënt schreef, verwezenlijkt.
Dr. J.L. Willems; beste Hans, je betrokkenheid bij het onderzoek en je kalme optimistische benadering van onze analytische problemen hebben bij mij de stellige overtuiging doen postvatten dat er geen noot zo hard is of hij kan gekraakt worden, als maar gezocht wordt naar de juiste techniek.
Mevr. G.M. Vogels-Mentink; beste Trude, zonder jou zou dit proefschrift niet zijn voltooid. Je volhardende, kritische en nauwkeurige houding tijdens het opstarten van de methoden zijn van zeer grote waarde geweest. Met veel genoegen denk ik terug aan het werk in het laboratorium.
Prof. C. v. Os, Dr. R. Bindels en Dr. P. Vis wil ik graag bedanken voor hun kritische en stimulerende bijdragen aan de discussie omtrent geïoniseerd magnesium en de intracellulaire transportprocessen.
Prof. C. Hilbers, J.J.M. Joordens, Dr. G. Rijksen en Dr. R. Ouwerkerk ben ik zeer erkentelijk voor hun bijdrage bij het opstarten en uitvoeren van de ^{31}P NMR metingen.
Aan de dierexperimenten hebben Albert Peters, Leo Jansen, Theo Arts, Ton Peters en Fred Philipsen, allen medewerkers van het Centraal Dieren Laboratorium (hoofd: Prof.Dr. W.J.I. v.d. Gulden, Dr. J. Koopman) een onontbeerlijke bijdrage geleverd.
Theo de Boo en Wim Lemmens namen de statistische bewerking van de onderzoeksresultaten voor hun rekening en leverden een significante bijdrage aan de discussie omtrent de uitkomst.
Cees Nicolasen verzorgde vele van de tekeningen.
De staf en medewerkers van het Centraal Klinisch Chemisch Laboratorium en het Laboratorium Kindergeneeskunde en Neurologie verleenden ons gastvrij onderdak tijdens het onderzoek en verrichtten vele hand- en spandiensten.
Vele medewerkers en medewerksters van de kinderkliniek hebben bijdragen geleverd aan de uitvoering van de magnesium titratie en resorptietesten evenals de selectie van patiënten voor de referentiewaarden.
De leden van de secretariële groep Kindergeneeskunde evenals Jeroen Hopman leverden ondersteuning in de verschillende fasen van voorbereiding en afwerking van dit proefschrift.
Mijn collegae neonatologen dank ik voor hun flexibiliteit en bereidheid taken over te nemen tijdens de periode van het onderzoek en op schrift zetten van de resultaten.
Beste Lya; "zonder woorden" is veelzeggend.

Het is helaas onmogelijk ieder die een bijdrage leverde aan het tot stand komen van dit proefschrift hier te noemen; ook zij die niet werden vermeld dank ik hartelijk.
Niet in de laatste plaats ben ik allen die een bijdrage leverden aan mijn medische "opvoeding", zeer erkentelijk.

CURRICULUM VITAE

De auteur van dit proefschrift werd op 31 januari 1951 te Dinxperlo geboren.
In 1967 behaalde hij het MULO A diploma aan de RK ULO school te Silvolde, gevolgd door het HBS-B diploma aan het Sint Ludgercollege te Doetinchem in 1970.
De studie geneeskunde aan de Katholieke Universiteit Nijmegen werd in 1979 afgesloten met de bevordering tot arts.
Daarna was de auteur gedurende een jaar als arts-assistent kindergeneeskunde werkzaam in de praktijk van A.A.J. Broekman en J. Vonk, kinderartsen, Juliana Ziekenhuis respectievelijk St. Josef Ziekenhuis te Zaandam.
Van januari 1981 tot 1985 werd de opleiding tot kinderarts gevolgd in het St. Radboud Ziekenhuis te Nijmegen (Opleiders: Prof. Dr. E.D.A.M. Schretlen en Prof. Dr. G.B.A. Stoelinga).
Een fellowship neonatologie werd vervuld van 1985 tot 1987 op de subafdeling neonatologie van het St. Radboud Ziekenhuis te Nijmegen (hoofd: Dr. J.M. Boon).
Sindsdien is de auteur als kinderarts/neonatoloog verbonden aan laatst genoemde afdeling.
De auteur is gehuwd met Lya Boere en samen hebben zij drie dochters; Leontien, Elisabeth en Barbara.

STELLINGEN

behorend bij het proefschrift

MAGNESIUM METABOLISM IN CHILDHOOD

W.B. Geven

STELLINGEN

I

Een verlaagde concentratie van magnesium in het serum gaat niet gepaard met een verlaging van het totale magnesium gehalte in mononucleaire cellen.

Dit proefschrift.

II

Het totale magnesium gehalte van erythrocyten is een betere weerspiegeling van het lichaamsmagnesium dan dit gehalte in de mononucleaire cel.

Dit proefschrift.

III

Het is met de huidige kennis niet steeds mogelijk het syndroom van Bartter te onderscheiden van het syndroom van Gitelman.

Dit proefschrift.

IV

Magnesium toediening aan patiënten met een vers hartinfarct leidt tot een significant hogere overleving en berust zeer waarschijnlijk op een farmacologisch effect.

M. Schechter. Magnesium-Bulletin. 1990;12:1-5.

V

Bij een eenzijdige longagenesie, welke gepaard gaat met expiratoire belemmering, dient ernstig rekening te worden gehouden met het bestaan van een tracheastenose.

T. Weber et al. Ann. Surg. 1991; 213: 70-74.

VI

Een uitgebreide deletie van het collageen 4α5-gen bij lijders aan het syndroom van Alport, predisponeert tot het ontstaan van een anti-GBM nefritis na transplantatie.

Alport workshop Oulu 1991.

VII

Bij het berekenen van het alveolair-arterieel zuurstof verschil ($AaDO_2$) dient men te beseffen dat het fysiologisch correcter is het eind-expiratoire CO_2-gehalte in de formule in te vullen in plaats van de arteriële CO_2-spanning.

IX

De resultaten van de toediening van exogeen surfactant aan prematuren met het "Respiratory Distress Syndrome" zijn zo evident dat deze medicatie niet meer kan worden onthouden, ondanks de te verwachten budgettaire problemen.

X

Bij kinderen met cataract en chronische diarree moet cerebrotendineuze xanthomatose (CTX), middels bepaling van gal-alcoholen in de urine, worden uitgesloten.

J.R.M. Cruysberg et al. Am. J. Ophtalmol. 1991; 112: in press.

XI

De ontrafeling van de moleculaire basis van osteogenesis imperfecta begint het mogelijk te maken genotype en phenotype te correleren.

P.H. Byers et al. J. Med. Genet. 1991; 28: 433-443.

XII

De beste manier om tijdschriften op het gebied van de kindergeneeskunde in ongekreukte toestand te kunnen bewaren, is ze op te bergen in een bibliotheek op afstand.

XIII

Het ontbreken van (voldoende) facultaire erkenning voor het klinisch chemisch vakgebied leidt op termijn tot een daling van de kwaliteit van de klinisch chemische dienstverlening ook in niet academische ziekenhuizen.

XIV

Voor diegenen die een (huwelijk)relatie aangaan met een medicus(m/v) is Hall's artikel "Medical marriage: no bed of roses" aanbevolen literatuur.

A. Hall. B.M.J. 1988; 296: 152-153.

ISBN 90-9004412-4