Diagnosis of Magnesium Status

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Zusammenfassung

Weibliche Sprague-Dawley-Ratten erhielten über 12 Tage Diäten mit ansteigendem Mg-Gehalt (Faktor 6), um alimentär Mg-Mangel (250 ppm Mg), ausreichenden Mg-Versorgung (1500 ppm Mg) und Mg-Überschuß (9000 ppm Mg) zu induzieren. Folgende Parameter spiegelten diese drei Bedingungen signifikant wider: Plasma-Mg, Knochen-Mg, 6h-Urin-Mg (stimuliert durch Wasser und durch Wasser plus Furosemid-Diurese) und die Mg:Creatinin-Ratio im Urin. Die Tatsache, daß intrazelluläre Mg-Spiegel nicht mit ansteigender alimentärer Mg-Zufuhr korrelierten, wird diskutiert. In Verbindung mit 6h-Urin-Mg erwies sich der akute orale Mg-loading-Test von untergeordnetem Wert.

Im zweiten Teil der Arbeit wurden verschiedene Gewebe und wiederum 6h-Urin dahingehend untersucht, ob sie für die Erfassung eines marginalen, alimentären Mg-Mangels (250 ppm Mg im Futter) geeignet sind. Wiederum wurden die Mg-Diäten 12 Tage an Ratten verfüttert, als Kontrolle dienste die 1500 ppm Mg-Diät. Für Plasma-Mg, Knochen-Mg und 6h-Urin-Mg (sowohl nach akuter oraler Gabe von Wasser allein als auch in Verbindung mit Furosemid) ergaben sich höchst signifikante Unterschiede zwischen Mg-Mangel und Kontrollgruppe, während sie für Erythozyten-Mg schwach siginifikant ausfielen. Herzmuskel-Mg und Skelettmuskel-Mg dagegen zeigten keine Veränderungen durch marginale Mg-Zufuhr.

Summary

Groups of female Sprague-Dawley rats were fed diets enriched with increasing (factor of 6) amounts of magnesium (Mg) during 12 days to produce marginal deficiency (250 ppm Mg), normal status (1500 ppm Mg) and Mg-excess (9000 ppm Mg). Parameters significantly correlating with these three conditions were found to be: plasma-Mg, bone-Mg, 6h-urine-Mg (stimulated by water and water plus furosemide diuresis) and the urinary Mg: creatinine ratio. Probably due to saturation processes, intracellular Mg levels did not correlate with increasing dietary magnesium intake. 6h-urine-levels measured after acute oral water plus Mg loading proved to be of minor diagnostic value. In part 2 of the study Mg-concentrations measured in different tissues and in 6h-urine were examined as tools to detect Mg-deficiency in rats pretreated during 12 days with a moderately Mg-deficient diet (250 ppm Mg) in comparison to controls (1500 ppm Mg). Mg-concentrations measured in plasma, bone and in 6h-urine (stimulated by loading with water alone or in combination with furosemide) yielded best and highly significant results ($p<0.001$), followed by erythrocyte-Mg ($p<0.05$). On the other hand neither myocardial-Mg nor skeletal muscle-Mg reflected marginal Mg-deficits.

Résumé

Des groupes de rats femelles Sprague-Dawley ont reçu, pendant 12 jours, une nourriture enrichie par des quantités croissantes (selon un facteur 6) de magnésium (Mg), créant ainsi un régime légèrement carencé (250 ppm Mg), normal (1500 ppm Mg) ou excédentaire en magnésium (9000 ppm Mg). Les paramètres présentant une corrélation significative avec cet accroissement de l'apport alimentaire de Mg ont été les concentrations plasmatiques, osseuses et urinaires (urines de 7h stimulées par une charge hydrique supplémentée ou non par du furosémide) de magnésium, ainsi que le rapport urinaire magnésium (créatinine. En raison vraisemblablement de processus de saturation, les concentrations intra-cellulaires de Mg n'ont pas été corrélées à l'augmentation du Mg alimentaire. Les concentrations des urines de 7h mesurées après charge hydrique orale aiguë supplémentée en Mg se sont avérées avoir une faible valeur diagnostique. Au cours de cette partie de l'étude, les auteurs ont recherché si la mesure des concentrations de Mg dans différents tissus et dans les urines de 6h permettait de déceler une carence en Mg chez des rats pré-traités pendant 12 jours par un régime alimentaire modérément hypomagnésié (250 ppm Mg) ou normal (témoins 1500 ppm Mg). La meilleure valeur diagnosti que a 'té obtenue par la mesure des concentrations plasmatiques, osseuses et urinaires (urines de 6h stimulées par une charge hydrique supplémentée ou non par du furosémide) de magnésium dont les résultats ont été hautement significatifs ($p<0,001$), suivies par les concentrations intra-crythrocytaires ($p<0,05$). En revanche, les mesures du Mg dans le myocarde et le muscle squelettique n'ont pas parmis de déceler une faible carence en magnésium.

1. Parameters Correlating with Experimentally Induced Deficiency and Excess

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Introduction

In practical medicine a discrepancy still exists between the well documentated importance of magnesium (Mg) in metabolic processes correlated with clinical symptoms of Mg-deficiency, being frequently recognised, and a missing unambiguous parameter for routine Mg diagnostics (*Seelig,* 1981; *Wester,* 1982; *Classen,* 1986; *Classen,* 1987). In nephrology, on the other hand, severely impaired renal function induces Mg accumulation, which may also be associated with adverse clinical symptoms (*Berlyne,* 1972; *Cat-*

to, 1974). Difficulties in laboratory diagnosis mainly originate from the unfavourable distribution of Mg in the organism, and, in addition, alternating interrelations may exist between different compartments (*Fischer,* 1981; *Elin,* 1987a; *Classen,* 1990). Furthermore methodical difficulties still exist to determine extra- and intracellular free Mg^{2+} which seems to be the biochemically active fraction of Mg (*Speich,* 1981; *Elin,* 1987b) and hence is thought to be a better indicator of Mg-status than total Mg.

Nevertheless, total serum-/plasma-Mg still is the main parameter for Mg diagnosis in practical medicine since it can be obtained easily and measured rapidly and precisely using the atomic absorption spectrophotometry technique (AAS). However, serum-plasma-Mg only represents ca. 0.3 % of total body Mg. Although hypomagnesemia (at normal albumin concentrations) unequivocally reflects Mg-deficiency, normomagnesemia does not exclude it (*Whang,* 1987). Acute stress, for example, leads to an influx of cellular Mg into the vascular pool (*Joborn,* 1985). Also, exchangeable bone-Mg can maintain normomagnesemia at intracellular deficits for certain periods of time.

Erythrocyte-Mg represents another Mg-parameter easy to obtain, with the advantage of representing intracellular Mg. But a standardised method to measure erythrocyte-Mg is not generally accepted and, in addition, erythrocytes are free of nuclei and their Mg-content also depends on genetic factors (*Gueux,* 1988; *Lasserre,* 1989) and on their age (*Elin,* 1980).

Bone-Mg comprising about 60 % of total body Mg represents the main Mg-pool. In vivo studies showed an exchangeable bone-Mg-pool of about 14–35 % in man (*Alfrey,* 1973) and of about 20–70 % in young rats (*Wallach,* 1988). These exchangeable pools may serve as buffers to maintain Mg-homeostasis. Therefore bone-Mg should be included in balance-studies. Some authors stress on skeletal muscle-Mg as a suitable parameter to detect Mg-deficiency in man (*Caddell,* 1967; *Lim,* 1972), whereas cardiologists point out the importance of measuring myocardial-Mg, e. g. in cases of infarction or coronary heart disease (*Speich,* 1980; *Ralston,* 1989).

The fact that Mg-homeostasis is mainly regulated by the kidneys explains why urinary Mg excretion has been studied intensively. 24h-urine-Mg levels as well as parenteral and oral Mg-loading tests, resp. retention-tests, combined with two 24h-urine sampling periods are often recommended in clinical medicine to assess the Mg-status (*Seelig,* 1980; *Durlach,* 1988). However, besides interfering effects on 24h-urine-Mg, such as nutrition, hormonal regulation and exercise, and the necessity to perform parenteral loading tests only under clinical control it must be considered that sampling of 24h-urine per se is difficult to realize, especially in practical medicine (*Ryzen,* 1985; *Caddell,* 1989).

With these data in mind it seemed to be worthwhile to reevaluate different parameters discussed above under precisely controlled conditions. Our main purpose was to shorten the time of urine collection to periods which are acceptable under practical clinical conditions. For this purpose the production of urine had to be stimulated. The following methods were applied:

— stimulation by water-loading
— stimulation by water-loading plus furosemide*
(*Reyes,* 1982; *Leary,* 1984; *Ryan,* 1984)
— stimulation by water-loading plus Mg.

Part 1 of these studies is concerned to find out suitable parameters correlating with the actual Mg status (including deficiency as well as excess), whereas part 2 will deal with parameters suitable to detect (dietary) Mg-deficiency.

Materials and Methods

Experimental design

81 female Sprague-Dawley rats (Interfauna, Tuttlingen, FRG), initially weighing about 100 g, housed in individual cages under controlled conditions, were fed three different diets to induce deficiency, normal status, and excess, respectively. For this purpose, a Mg-deficient semipurified powdered diet (Altromin C1035, Lage, FRG; 112 ± 9 ppm Mg, n = 5 samples, determined by analysis) served as basal diet which was enriched with Mg-aspartate-HCl (Verla-Pharm, Tutzing, FRG) to yield final concentrations of 250 ppm, 1500 ppm and 9000 ppm Mg. Diets and demineralised water were offered ad libitum during 12 days. Then, following an overnight fast, the animals were transferred to metabolism cages. The test solutions:

H_2O = 2 x 25 ml/kg b.w. water
FURO = 50 mg/kg b.w. furosemide in 2 x 25 ml/kg b.w. water
Mg = 250 mg/kg b.w. Mg in 2 x 25 ml/kg b.w. water

were applicated intragastrally twice at times 0 h and 3 h. After sampling urine for 6 h, blood was withdrawn by punction of the aorta abdominalis and organs were taken for analysis under pentobarbital i.p. anaesthesia (Nembutal®, CEVA, FRG).

Analysis

Magnesium was generally determined by AAS-technique (Perkin Elmer 1100) after appropriate dilution.

Organs: Specimens were lyophilisised for 48 h and ashed twice for 24 h (550° C); electrolytes were measured in aqueous solutions containing 7.6 g/l $SrCl_2$.

Erythrocytes were washed twice in cold 10.8 % sucrose and then lysed with bidistilled water.

Creatinine: To guarantee unimpaired renal function plasma- and urine-creatinine concentrations were determined by the modified method of Jaffé (Merckotest 3385, Merck, FRG).

Statistics

Combining the 3 diets [250, 1500, 9000 ppm Mg] and the 3 methods to stimulate urine excretion [H_2O, *FURO*, *Mg*] a 3^2-factorial design was chosen for randomisation. Since 9 repetitions were necessary for technical reasons, the 81 rats were randomly allocated to a 9 x 9 Latin Square design.

* GB: frusemide.

To evaluate possible effects of diets and treatments either on single parameters or on the entity of all parameters, univariate and multivariate analysis of variance were used. A possible influence of body weight was tested by analysis of covariance. Regression analysis was applied to test the dependency of different parameters from dietary Mg supply. Relations between single parameters were tested by analysis of correlation. SAS® (SAS, 1988) was used for all statistical procedures (ANOVA, GLM, REG, CANCORR).

Results

During the 12 days feeding period the rats did not exhibit clinical signs of Mg-deficiency like erythema or increased excitability. Hence, only "marginal" Mg-deficiency was induced, as intended. Animals kept on 9000 ppm Mg developed some stool-softening during 3 to 4 days, however, diarrhoe then disappeared.

At the end of the feeding period body weights were significantly lower in the 9000 ppm groups (–7 %; $p < 0.001$). However, analysis of covariance revealed that the body weight did not affect the parameters under study.

No mortality occurred during the whole experiment.

Plasma-creatinine revealed no impaired renal function in any of the groups. Urine-creatinine was also within the normal range and was used to calculate the ratio of magnesium: creatinine [mmol:mmol] (see fig. 1).

6 h-urine-Mg

Generally, 6 h-urine-Mg significantly reflected variations of dietary Mg.

With respect to the three different treatments applied, no overt differences existed between H_2O- and *FURO*-stimulated urinary Mg-**concentrations** [mmol/l]. 6 h-urine-Mg-concentrations only increased significantly following *Mg*-loading. However, high variations occurred; therefore the effect of the different diets was less significant under these conditions (see fig. 2).

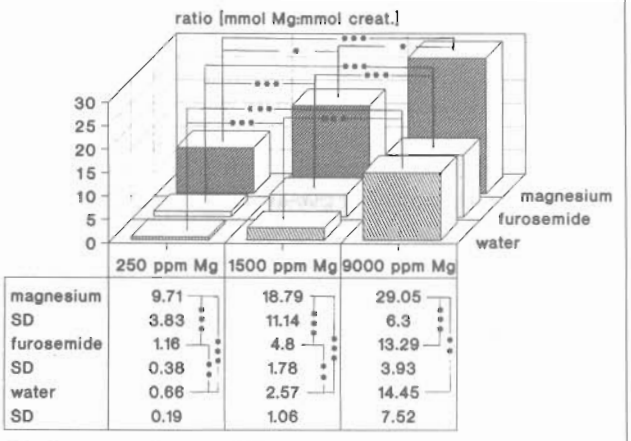
If **amounts of Mg in 6 h-urine** [µmol] were calculated, significant differences between H_2O- and *FURO*-induced 6 h-urine-Mg became obvious. As expected, the amounts of excreted Mg were lowest following H_2O-induced diuresis and highest after *Mg*-loading (see fig. 3).

The magnesium:creatinine-ratio, shown in fig. 1, closely corresponds to 6 h-urine-Mg [µmol]: That means, differences in dietary Mg-intake and in urine-producing treatments can be ascertained. In addition, significant deviations of urine-creatinine can be excluded, that is renal function was not affected by any treatment.

Detection of variations according to different Mg-supply

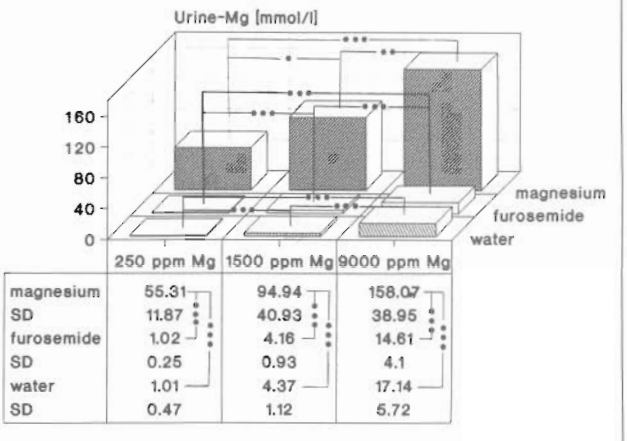
To evaluate the validity of the different parameters measured for the assessment of the actual Mg-status and to compare the single parameters directly, linear regression analysis was applied. Logarithms of dietary Mg (ln250 = 5.5; ln1500 = 7.3; ln9000 = 9.1) served as independent variable (x-axis, Mg-status).

Since absolute Mg-concentrations of different parameters greatly differed (i.e. plasma-Mg ca. 1.0 mmol/l; bone-Mg ca. 200 mmol/kg d.w.) data of the y-axis were transformed into comparable dimensions. Using the equations of the respective regression lines percental alterations of the Mg-content between two diets (ln1500/ln9000) were calculated taking the value of ln1500 (control) as 100 % (fig. 4–7).



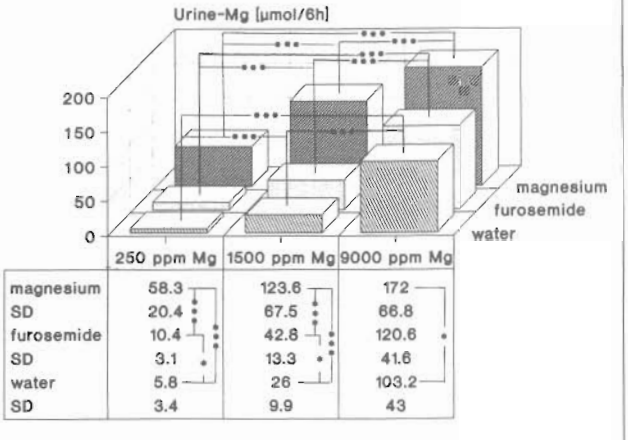
	250 ppm Mg	1500 ppm Mg	9000 ppm Mg
magnesium	9.71	18.79	29.05
SD	3.83	11.14	6.3
furosemide	1.16	4.8	13.29
SD	0.38	1.78	3.93
water	0.66	2.57	14.45
SD	0.19	1.06	7.52

Fig. 1: magnesium:creatinine-ratio of 6h-urine [mmol:mmol] SD = standard deviation



	250 ppm Mg	1500 ppm Mg	9000 ppm Mg
magnesium	55.31	94.94	158.07
SD	11.87	40.93	38.95
furosemide	1.02	4.16	14.61
SD	0.25	0.93	4.1
water	1.01	4.37	17.14
SD	0.47	1.12	5.72

Fig. 2: 6h-urine-Mg-concentration [mmol/l] SD = standard deviation



	250 ppm Mg	1500 ppm Mg	9000 ppm Mg
magnesium	58.3	123.6	172
SD	20.4	67.5	66.8
furosemide	10.4	42.8	120.6
SD	3.1	13.3	41.6
water	5.8	26	103.2
SD	3.4	9.9	43

Fig. 3: amounts of urine-Mg in 6h [µmol/6h] SD = standard deviation

In agreement with the data gained by regression analysis, Figures 4–7 clearly demonstrate that plasma-Mg as well as bone- and urine-Mg strongly depend on the actual Mg-status. Acute loading with *Mg* revealed again a decreasing influence on plasma- and urine-Mg. It should be noted that erythrocyte-Mg was only slightly affected following loading with H_2O, whereas in the *FURO*- and in the *Mg*-loaded groups erythrocyte-Mg as well as skeletal muscle- and myocardial-Mg remained completely uneffected (see tab. 1).

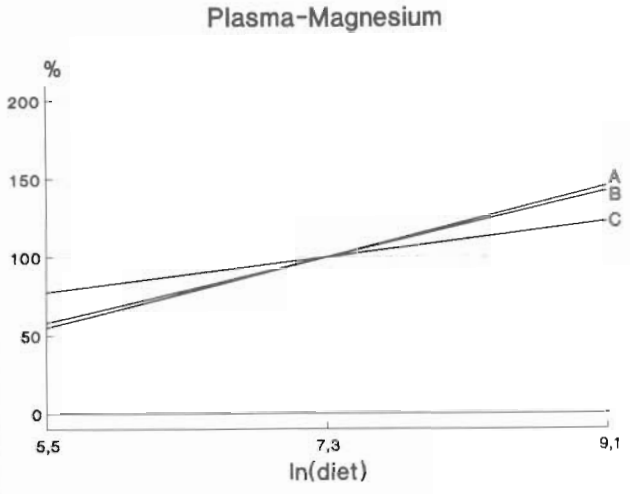
Finally, correlation analysis should reveal possible relationships between different Mg-parameters. In tab. 2, only highly significant correlations with $p < 0.001$ are summarised. According to their role in Mg-homeostasis, plasma-, bone- and urine-Mg showed good correlations. However, no significant correlations were found with erythrocyte-Mg, skeletal muscle- and myocardial-Mg.

Discussion

The present study was designed to find out suitable parameters for the discrimination of different states of Mg-balance, induced in rats by feeding three diets with different Mg-concentrations during a period of time long enough to achieve steady-state conditions. Standard feed pellets for rats usually contain ca. 2000 ppm Mg, the requirement of rats however amounts to only 400 ppm Mg (*Rogers*, 1979). With these data in mind the chosen experimental diets (250 ppm Mg, 9000 ppm Mg) represent only moderate deficiency, resp. excess. Hence, after 3–4 days of adaptation, the different groups were not discernible by clinical signs like behaviour.

Urine

Urine was collected during only 6 h and production had therefore to be stimulated by the administration of



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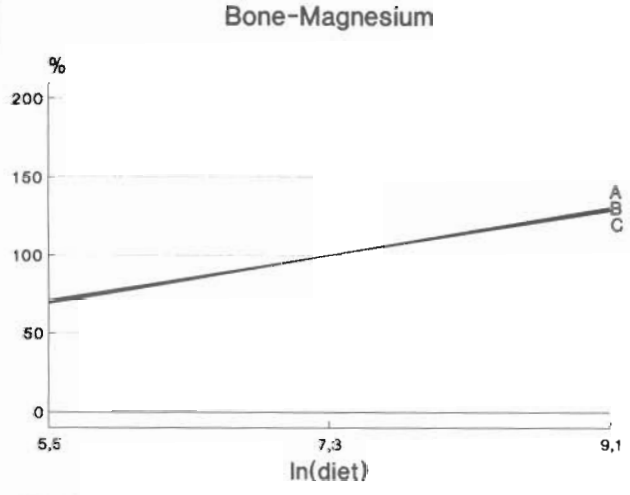

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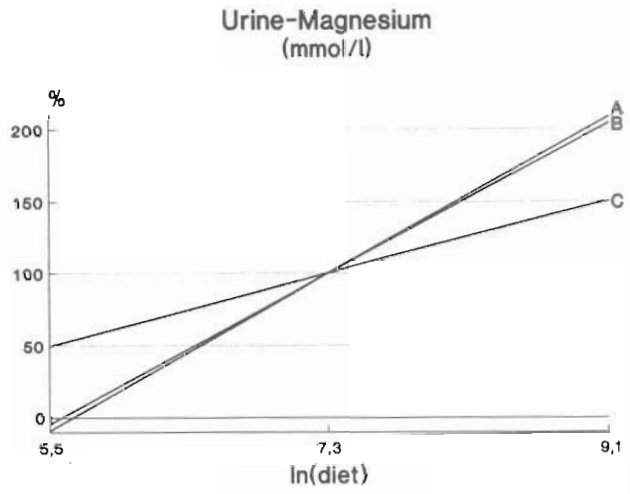

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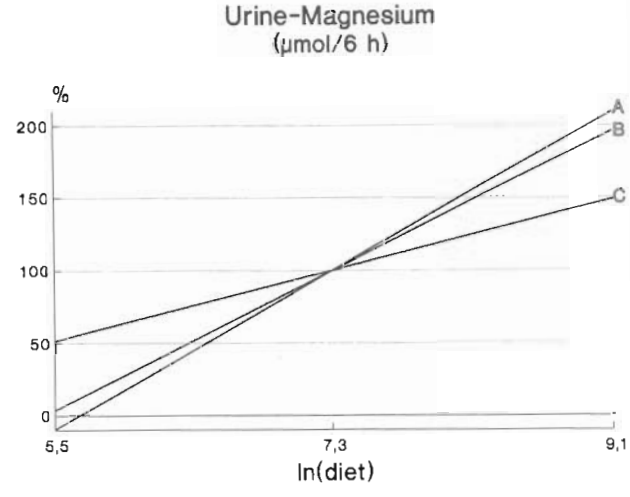

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Fig. 4–7: Regression analysis with diets as independent variables and Mg-parameters as dependent variables, subdivided as well with treatments. Transformation of the axis:
x-axis: ln of Mg-content of the given diets, that means: ln250=5.5, ln1500=7.3, ln9000=9.1
y-axis: Percental alteration compared to control (1500 ppm Mg in diet); for details see text
A = H_2O B = *FURO* C = *Mg*

Tab. 1: Regression analysis
First line indicates square correlation (r^2) between Mg-content of diets (as ln) and Mg-parameters with corresponding significance.
Second line indicates the equation of regression line: significance refers to slope of regression line.
*** = p<0.001 * = p>0.05
n = 27

	treatment		
	H_2O	FURO	Mg
femur	0.885 *** -32.0+30.8x ***	0.834 *** -35.9+30.8x ***	0.931 *** -48.3+33.6x ***
plasma	0.921 *** -0.68+0.21x ***	0.920 *** -0.57+0.19x ***	0.660 *** 0.14+0.17x ***
urine [mmol/l]	0.742 *** -25.4+4.5x ***	0.789 *** -21.4+3.8x ***	0.633 *** -106+28.7x ***
urine [µmol/6h]	0.675 *** -153+27.2x ***	0.747 *** -166+30.7x ***	0.434 *** -114+31.7x ***
erythrocyte	0.229 * 1.4+0.1x *	0.078 1.9+0.1x	0.082 2.0+0.1x
skeletal muscle	0.114 37.3+0.9x	0.002 42.4-0.2x	0.041 39.9+0.5x
myocard	0.046 30.8+0.6x	0.036 40.1-0.7x	0.109 31.4+0.7x

Tab. 2: Correlation analysis between Mg-parameters
r = coefficient of correlation
Limit of significance was p<0.001 which corresponds to |r| > 0.597 with n = 27

	treatment		
r	H_2O	FURO	Mg
plasma/femur	0.960	0.909	0.824
plasma/urine [mmol/l]	0.891	0.897	0.605
plasma/urine [µmol/6h]	0.792	0.846	n.s.
femur/urine [mmol/l]	0.868	0.900	0.842
femur/urine [µmol/6h]	0.772	0.852	0.654
urine [mmol/l] / urine [µmol/6h]	0.938	0.920	0.760
urine [mmol/l]/ratio	0.923	0.955	0.812
urine [µmol/6h]/ratio	0.783	0.913	0.853

water, which will also be possible under clinical conditions.

Mg-loading: It was assumed that Mg-retention was more intensive in deficient rats than in animals receiving excessive Mg in their diets. However, this procedure proved to be rather unsuitable under the chosen conditions, certainly because no steady-state conditions were achieved within 6 h: Hypermagnesemia was observed in all 3 groups (independent from diets) and femur-Mg remained unchanged (see fig. 4 and 5). Dose-response-curves between dietary Mg and urine-Mg (concentrations or total amounts excreted) exhibited only a weak rise of the curve (see fig. 6 and 7) and are therefore unsuitable for diagnosis. With regard to clinical medicine, two factors should be mentioned in this connection: first, patients should not take Mg-rich meals right before (say 12 h) samples are taken for analysis and second, studies concerned with the therapeutic availability of orally administered Mg salts, being usually estimated by the amounts of Mg excreted via urine (Lücker, 1985), must provide "optimal" periods of urine collection and should be done only at comparable filling-status of deep compartments like bone.

Treatment with *H_2O* or *FURO* yielded favourable dose-response effects (see fig. 6 and 7). Concerning **6h-urine-concentrations** (mmol Mg/l urine) both treatments yielded similar results (fig. 6). Concerning **amounts of urine-Mg** excreted, that is taking urine-volume also into consideration (µmol Mg in 6h), it becomes evident that *FURO* somewhat increased Mg-excretion (see fig. 7). This fact is probably due to increased urine-volume stimulated by *FURO:* In 1936, *Brull* already achieved Mg-depletion of dogs after intragastral application of increasing amounts of distilled water. — Nevertheless, these data confirm Mg-losses following longterm therapy with loop diuretics in man (*Lim*, 1972).

Some authors prefer the ratio of magnesium : creatinine in urine as parameter, especially in childhood (*DeSanto*, 1988; *Shaw*, 1990). Shaw, for example, used this ratio in connection with only one urine sample which surely is a technical advantage in children. In our study this parameter sufficiently reflected the effect of different diets and of the test-substances applicated, that means the ratio closely corresponds to amounts of 6h-urine-Mg. However, it must be noted that urine-creatinine did not show greater variations which would of course render this parameter less meaningful.

The present study again confirms the validity of **plasma-Mg**, at least in the assessment of alimentary effects on Mg-status: Plasma-Mg significantly reflected Mg-status — induced by diets as shown by steep slopes of the respective regression curves (fig. 4). As mentioned already acute *Mg*-loading induced hypermagnesemia in all groups, independently of the diet. Therefore the slope flattens (see fig. 4) —. According to *Fischer*, 1981, *Mutschler*, 1982, and *Elin*, 1987b, plasma-Mg does not reflect Mg-concentrations of other tissues. But, in agreement with the results of *Alfrey*, 1974, we found high correlations with bone-Mg, the main Mg-pool, which supports the validity of plasma-Mg.

According to our results **bone-Mg** would also provide a suitable parameter to detect longterm variations of Mg-status — since the Mg-content of femora reflected Mg-deficiency as well as Mg-excess (fig. 5). Neither acute *Mg*-loading nor treatment with *FURO* affected bone-Mg. In addition, the central role of bone-Mg-pool could be confirmed by the expected high correlations with plasma- and urine-Mg. However, as a routine diagnostic parameter in man, bone-Mg has the great disadvantage that an operative biopsy is necessary often yielding little and inhomogenous material. If, however, biopsies are performed in the course of other treatments samples should be taken whenever possible to get more

information about the actual Mg-status. Attention must however be paid to the fact that different types of bone may contain different concentrations of Mg and also that different types of bone respond to different Mg-supply within different time-intervals (*Wallach*, 1987; *Durlach*, 1988, *Beier*, 1991).

Tissue-Mg

Erythrocyte-Mg only slightly correlated with dietary Mg. Skeletal muscle-Mg and myocardial-Mg exhibited neither dependency on diets nor correlations with other compartments. Hence within the broad range of Mg-supply studied these parameters are not predictive certainly because of saturation processes. This does not exclude the possibility that tissue-Mg concentrations reflect Mg-deficiency. This possibility will be discussed in part 2 of this study.
Finally, it should be pointed out that dietary factors greatly affect Mg-balance. Hence, a careful dietary anamnesis must be included in the diagnosis of the actual Mg-balance.

2. Parameters Sensitive to Detect Experimentally Induced Magnesium Deficiency

Introduction

In part 1 of these experiments, parameters were evaluated which are suitable to reflect the actual Mg-status over a large concentration scale, including deficiency and excess.
Part 2 of the study will stress only on Mg-deficiency which certainly occurs more frequently and hence is more important under clinical conditions (for summary see *Classen*, 1986).

Materials and Methods

Experimental design

For a detailed description see part 1 of this study. In short, two subgroups of young female Sprague-Dawley rats were fed diets containing 250 ppm and 1500 ppm for 12 days to induce deficiency and well-balanced Mg-status. Following an overnight fast the animals were transferred to metabolism cages and the following test solutions were applicated intragastrically:

H_2O = 2x25 ml/kg b.w. water;

FURO = 50 mg/kg b.w. furosemide in 2x25 ml/kg b.w. water.

After sampling urine for 6h withdrawal of blood was done by punction of the aorta abdominalis and organs were taken under pentobarbital-anaesthesia (Nembutal®, CEVA, FRG). Since acute *Mg*-loading did not yield satisfying results, these data are not included.

Analysis

Generally, Mg and Ca were determined by using the AAS-technique (Perkin Elmer 1100).

K was determined by emission spectrophotometry.

Erythrocytes were washed twice in cold 10.8% sucrose and then lysed with water.

Organs: Specimens were lyophilisised for 48h and ashed twice for 24h (550° C) before measuring electrolyte content.

Statistics

To evaluate influence of the two diets and the two treatments (H_2O and *FURO*) on single parameters, univariate analysis of variance was used even to compare Mg-deficient and control groups (1500 ppm Mg) because the two different treatments were again involved in evaluation. Relationships between single parameters were tested by analysis of correlation. SAS® was used for all statistical procedures (ANOVA, CANCORR) (SAS, 1988).

Results

Detecting Mg-deficiency

For detecting Mg-deficiency percental deviations were calculated between the groups fed 250 ppm Mg and 1500 ppm Mg. In addition, effects of treatment with H_2O und *FURO* have been evaluated.
Plasma-Mg-levels averaging to 0.47 or 0.48 mmol/l in the 250 ppm Mg subgroups reflect Mg-deficiency in comparison to the generally accepted normal range of 0.75–1.10 mmol/l. Plasma-Mg-levels averaging to 0.82 or 0.84 mmol/l in the 1500 ppm Mg subgroups ascertain that this group can be used as control group (tab. 3 and 4). The highly significant differences of plasma-Mg, femur-Mg and 6h-urine-Mg measured in Mg-deficient and control groups confirm the statement of part 1, that these parameters are suitable tools in Mg-diagnostics. — Erythrocyte-Mg revealed differences of about 18 % or 17 % between Mg-deficient rats and control groups, however these effects were remarkably less pronounced than changes occurring in plasma-, femur- or 6h-urine-Mg. One reason might be the high variation associated with the method used. In comparison to proposed normal ranges for erythrocyte-Mg of 1.8–2.8 mmol/l (*Durlach*, 1988), our values (2.07 or 2.33 mmol/l Mg in the Mg-deficient groups) did not detect Mg-deficiency (fig. 8 and 9).
Again myocardial- and skeletal muscle-Mg were not significantly affected by the two diets or treatments.
As in part 1 of the study correlation analysis was done within the 250 ppm and 1500 ppm Mg groups to detect relations between different Mg-parameters.
Again, the three Mg-compartments connected directly with each other, namely plasma-, bone- and urine-Mg exhibited highly significant correlations. On the other hand, erythrocyte-Mg, although significantly differing between the two groups, did not correlate with the other Mg-parameters (tab. 5).
The same was true for myocardial- and skeletal muscle-Mg.

Tab. 3: Percentage deviation between 250 ppm Mg- and 1500 ppm Mg-groups (H_2O-treatment).
1500 ppm Mg-groups (control) = 100 %
Significances originate from analysis of variance
*** = p<0.001 * = p<0.05

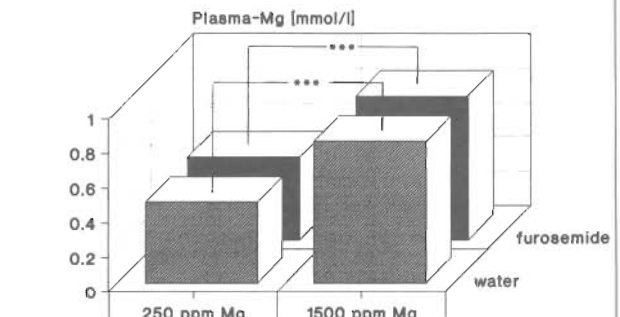
	H_2O treatment			
	250ppm Mg	1500ppm Mg	%	p
Plasma-Mg	0.47±0.07	0.82±0.06	42	***
Ery-Mg	2.07±0.30	2.54±0.44	18	*
Femur-Mg	139.2±14.1	191.6±14.5	27	***
Myocardial-Mg	33.7± 3.4	36.5± 1.1	4	n.s.
Muscle-Mg	41.9± 5.2	43.8± 2.8	7	n.s.
6h-urine-Mg [mmol/l]	1.01±0.47	4.37±1.12	76	***
6h-urine-Mg [µmol/6h]	5.8± 3.4	26.0± 9.9	77	***

Tab. 4: Percentage deviation between 250 ppm Mg- and 1500 ppm Mg-groups (*FURO*-treatment).
1500 ppm Mg-groups (control) = 100 %
Significances originate from analysis of variance
*** = p<0.001 * = p<0.05

	FURO treatment			
	250ppm Mg	1500ppm Mg	%	p
Plasma-Mg	0.48±0.05	0.84±0.05	42	***
Ery-Mg	2.23±0.38	2.69±0.27	17	*
Femur-Mg	134.4±12.6	180.5±22.4	23	***
Myocardial-Mg	33.6± 2.7	34.2± 6.9	1	n.s.
Muscle-Mg	41.8± 4.9	40.5± 7.2	3	n.s.
6h-urine-Mg [mmol/l]	1.02±0.25	4.16±0.93	75	***
6h-urine-Mg [µmol/6h]	10.4± 3.1	42.8±13.3	75	***

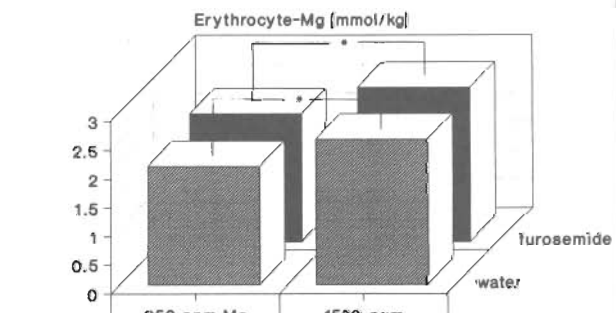
Tab. 5: Correlation analysis of 250 ppm Mg- and 1500 ppm Mg-groups between Mg-parameters
r = coefficient of correlation
Limit of significance was p<0.001 which corresponds to
|r| < 0.705 with n = 18

	treatment	
r	H_2O	*FURO*
plasma/femur	0.940	0.739
plasma/urine [mmol/l]	0.906	0.889
plasma/urine [µmol/6h]	0.834	0.841
femur/urine [mmol/l]	0.887	0.750
femur/urine [µmol/6h]	0.744	0.755
urine [mmol/l] / urine [µmol/6h]	0.909	0.954



	250 ppm Mg	1500 ppm Mg
furosemide	0.48	0.84
SD	0.05	0.05
water	0.47	0.82
SD	0.07	0.06

Fig. 8: Plasma-Mg [mmol/l]
SD = standard deviation



	250 ppm Mg	1500 ppm
furosemide	2.23	2.69
SD	0.38	0.27
water	2.07	2.54
SD	0.3	0.44

Fig. 9: Erythrocyte-Mg [mmol/l]
SD = standard deviation

Tab. 6: Correlation analysis including all kinds of diets (250, 1500 and 9000 ppm Mg) between Mg-parameters and secondary electrolytes (Ca, K).
r = coefficient of correlation
Limit of significance was p<0.001 which corresponds to
|r| < 0.597 with n = 27

	treatment	
r	H_2O	*FURO*
femur-Mg / plasma-Ca	-0.671	-0.598
plasma-Mg / plasma-Ca	-0.654	-0.645
myocardial-Mg / myocardial-K	0.889	0.903
muscle-Mg / muscle-K	0.803	0.862

Secondary electrolytes

As was expected, alterations in alimentary Mg-supply mainly affect Ca-homeostasis. The species-specific effect in rats of hypercalcemia occurring with hypomagnesemia became obvious and is shown by the significant negative correlation (see tab. 6).
In addition, obvious correlations exist between femur-Mg and plasma-Ca: Plasma-Ca was high at low femur-Mg and vice versa.
Although myocardial- and skeletal muscle-Mg did not significantly reflect the actual Mg-status good corre-

lations were found between Mg and K concentrations (tab. 6).

Discussion

As it was expected from part 1 **plasma-Mg, bone-Mg and 6h-urine-Mg after acute H_2O- or *FURO*- loading** turned out to be suitable tools to detect Mg-deficiency. Compared to the normal range of plasma-Mg of 0.75–1.10 mmol Mg/l (*Durlach,* 1988; *Classen,* 1990) diets providing only 250 ppm Mg induced marginal Mg-deficiency in rats.

In contrast to *Alfrey,* 1974, and *Geven,* 1988, who have described good correlation between plasma-Mg and **erythrocyte-Mg** (ery-Mg), we did not find any correlation between ery-Mg and other tissue-Mg levels neither in the experiments including Mg-excess (part 1) nor when only comparing Mg-deficient groups to controls. In the experiments discussed here and involving only Mg-deficient (250 ppm Mg) and normal control Mg-groups (1500 ppm Mg) ery-Mg was lowered only by about 17 % under Mg-deficiency at low levels of significance. These data indicate that Mg-metabolism in erythrocytes is slow, compared to other compartments, and also, that a certain degree of saturation may be achieved since levels did not further increase at dietary Mg-excess (see part 1). Our results are in accordance with the literature where the validity of ery-Mg in the assessment of Mg-status is discussed quite contradictory. One additional reason may be that no standardised methods are available. The method applied in our experiments revealed high variations of the single values ($r^2 = 0.08$ to 0.22). Additional reasons are the difficulties to separate reticulocytes from erythrocytes which have an 8fold higher Mg-content than erythrocytes, and the fact that ery-Mg decreases with age (*Elin,* 1980). Finally, ery-Mg is regulated also by genetic factors (*Gueux,* 1988; *Lasserre,* 1989) resulting in genetic polymorphism.

Some authors point out that **skeletal muscle-Mg** is an important parameter in Mg-diagnostics in man (*Caddell,* 1967; *Lim,* 1972). This is in contrast to our results. Muscle-Mg did not reflect dietary Mg-levels, and any correlations to other compartments could be established. *Hence,* our findings better agree with those published by *Robeson,* 1980, and *Wallach,* 1988, who reported unchanged muscle-Mg in Mg-deficient rats and with data of *Geven,* 1988 who did not find any effects in dogs. Species-specific effects might be involved (*Wallach,* 1987). For example, *Wallach* describes that the decrease observed in the Mg-concentration of rat muscle was small, within a range of 0–11 %, whereas in human muscle, Mg-deficiency led to a decrease of up to 40 %. It seems to be that above all in chronic Mg-deficiency skeletal muscle-Mg-pools will be included in Mg-homeostasis in man (*Lim,* 1972) but not in rats (provided that samples are not contaminated with serum). Nevertheless, the biopsy procedure excludes skeletal muscle-Mg as a routine parameter in Mg-diagnostics (*Elin,* 1987b).

Based on the fact that decreased Mg stores have been accused to favour myocardial damage and that myocardial-Mg was lowered in case of myocardial infarction, **myocardial-Mg** concentrations were also determined in this study. However neither any dependency on the different diets or treatments, nor any correlation of myocardial-Mg with other tissues could be demonstrated. It could be concluded that heart-Mg may be protected against Mg-loss at least for some time if no additional stress interferes. Similar results have been reported in man. *Ralston* (*Ralston,* 1989) conducted a study on 23 patients with symptoms of heart failure who received long-term diuretic treatment. Ralston, too, was unable to detect changes of myocardial-Mg. However, strong correlations were noted between Mg and K content in the tissues examined. The same was true in our experiments: Highly significant correlations could be established between Mg and K concentrations in myocardial and in skeletal muscle tissues, as well. Similar correlations have been reported by *Alfrey* (*Alfrey,* 1974). Hence, moderate Mg-deficiency represents a myocardial risk factor not per se, but rather due to secondary losses of potassium, favouring the development of fatal arrhythmia, for example.

In conclusion plasma-Mg as well as 6h-urine-Mg after stimulation with H_2O or *FURO* can be recommended as suitable parameters to detect Mg-deficiency under comparable conditions in practical medicine. These parameters are probably also suitable to monitor Mg-supplementations provided that steady-state conditions are obtained.

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