Biochemistry and Pathobiochemistry of Magnesium

By *Th. Günther*
Institut für Molekularbiologie und Biochemie, Freie Universität Berlin, Arnimallee 22, D-1000 Berlin 33

Zusammenfassung

Es werden die Wirkungen des Mg^{2+} auf die Kinetik und Thermodynamik enzymatischer Reaktionen, auf Nucleinsäuren und Phospholipide referiert.
Die Verteilung und der Stoffwechsel des Mg^{2+} in den verschiedenen intrazellulären Kompartimenten wird beschrieben. Das wesentliche Ergebnis ist, daß die intrazelluläre (Mg^{2+}) bei pMg 3 gepuffert ist.
Bei experimentellem Mg-Mangel ist die Zellmembranpermeabilität erhöht. Die intrazelluläre (Na^+), (Ca^{2+}) und (cAMP) sind angestiegen, die intrazelluläre (K^+) ist abgesunken. Die Wirkungen des intrazellulären Na^+ und Ca^{2+} und die regulatorischen Wirkungen des intrazellulären Ca^{2+} im Zusammenhang mit cyclischen Nucleotiden werden dargestellt. Die im Mg-Mangel gefundenen Änderungen der RNA, DNA, Protein- und Kollagenbiosynthese und des Energiestoffwechsels lassen sich auf diese Effekte zurückführen.
Im Mg-Mangel werden vermehrt Catecholamine freigesetzt. Dieser Effekt wird durch Streß verstärkt. Catecholamine verstärken die Wirkungen des Mg-Mangels durch ihre α- und/oder β-adrenergische Wirkung.
Die Wirkungen des Mg-Mangels werden am Versuchstier erst deutlich, wenn die Mg-Konzentration im Serum unter einen Schwellenwert von 0,7 mmol/l abgesunken ist.

Summary

The kinetic and thermodynamic effects of Mg^{2+} on enzyme reactions and the effects of Mg^{2+} on nucleic acids and phospholipids are reviewed. The distribution and metabolism of Mg^{2+} in the various intracellular compartments are described. The main result is that the intracellular [Mg^{2+}] is buffered at a level of pMg 3.
In experimental Mg deficiency, cell membrane permeability increases and intracellular (Na^+), (Ca^{2+}) and (cAMP) become elevated, whereas intracellular (K^+) decreases. The effects of intracellular Na^+ and Ca^{2+} and the regulatory effects of Ca^{2+} in connection with cyclic nucleotides are reviewed. The secondary alterations in RNA, DNA, protein and collagen biosynthesis and energy metabolism are explained by these effects. In Mg deficiency catecholamines are released to a higher extent, an effect that is magnified by stress. Catecholamines multiply the effects of Mg deficiency by an α- and/or β-adrenergic mechanism.
The effects of Mg deficiency in animals become only significant when the serum Mg^{2+} concentration has dropped below a threshold at 0.7 mmol/l.

Résumé

Nous rapportons les actions du Mg^{2+} sur la cinétique et la thermodynamique des réactions enzymatiques, sur les acides nucléiques et sur les phospholipides.
La répartition et le métabolisme du Mg^{2+} en les divers compartiments sont décrits. Le résultat essentiel est que le (Mg^{2+}) intracellulaire est tamponné à pMg = 3.
Dans le déficit expérimental en Mg, la perméabilité des membranes cellulaires est accrue. Les valeurs de (Na^+), (Ca^{2+}) et de l'(AMPc) intracellulaires sont accrues, le (K^+) intracellulaire abaissé. Nous présentons les actions du Na^+ et du Ca^{2+} intracellulaires et les actions régulatrices du Ca^{2+} intracellulaire en rapport avec les nucléotides cycliques. Les modifications trouvées, au cours du déficit magnésique, dans la biosynthèse des ARN, des ADN, des protéines et du collagène et dans le métabolisme énergétique peuvent être rapportées à ces effets.
Dans le déficit magnésique, les catécholamines sont libérées de façon renforcée. Cet effet est renforcé par le stress. Les catécholamines renforcent les actions du déficit magnésique par leurs actions α et/ou β-adrénergiques.
Les actions du déficit magnésique sur l'animal d'expérience ne sont manifestes que si la concentration en Mg dans le sérum est abaissée au dessous d'une valeur seuil de 0,7 mmol/l

Magnesium is an essential bioelement. About 95% of the magnesium content of organs is localized intracellularly. To define the role of Mg^{2+} in Mg deficiency, the main physiological effects of Mg^{2+} should be considered. In the final analysis, the biological effects of Mg^{2+} are caused by its ability to form chelates. In this connection, there is also a competition with Ca^{2+}. The chelate-forming properties of Mg^{2+} are related to substrates, enzymes and to substances of cell structures (e.g. proteins, phospholipids, nucleic acids). For review see [36].

Enzyme kinetic effects of Mg^{2+}

There are about 300 Mg^{2+}-activated enzymes. Their catalytic aktivity shows a bell-shaped pMg dependency (pMg = -log [Mg^{2+}], in analogy to pH, Fig. 1).
This means:

1. The catalytic activity increases with the logarithm of the concentration (ion activity) of free Mg^{2+}.
2. There is a pMg optimum in the range of pMg 3.
3. At the pMg optimum there is no change of the catalytic activity.

The bell-shaped pMg dependency consists of two sigmoid curves [18] viz.

I. Activation as a function of pMg
II. Inhibition as a function of pMg

The activation by Mg^{2+} results in the following general reaction patterns [91]

1. $Mg + S \rightleftarrows MgS$
   $MgS + E \rightleftarrows MgSE \rightarrow MgP + E$

where: S = substrate
E = enzyme
P = product

Mg^{2+} reduces the high negative charge of S, usually ATP^{4-} by chelate formation with two phosphate groups, especially the β and γP. [However, the exact structure of the Mg ATP complex is not established. The following complexes may exist in equilibrium:

$(MgP_\alpha P_\gamma)ATP^{2-} \rightleftarrows (MgP_\alpha P_\beta)ATP^{2-} \rightleftarrows (MgP_\beta P_\gamma)ATP^{2-}$.
The adenine ring is not involved in MgATP complex formation. For discussion and literature see [114].
MgS, the real substrate, is bound to the enzyme (e. g. hexokinase, phosphoglycerate kinase [26]).

2. $Mg + E \rightleftarrows MgE$
$MgE + S \rightleftarrows MgES \rightarrow MgE + P$

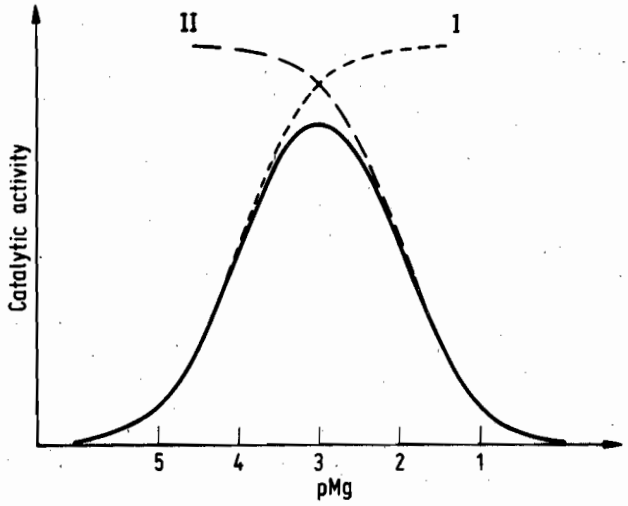

Fig. 1: Enzymatic activity of an Mg^{2+}-dependent enzyme as a function of pMg (schematic representation). I activation, II inhibition of the enzyme depending on pMg. From [36].

Mg^{2+} reacts with the enzyme, establishing its active conformation. In consequence, the substrate S is bound to the Mg enzyme complex (e. g. enolase, pyruvate kinase, pyrophosphatase [26]). Also in this case, Mg^{2+} may be involved in the binding of S. In both mechanisms, Mg^{2+} favours the reaction by additionally polarizing P-O bonds.
In special cases more than one Mg^{2+} must be bound (e.g. enolase must bind 4 Mg^{2+} for full activity [40]).

3. Both mechanisms may be combined, as is the case with F_1-ATPase of E. coli, Mg ATP being the real substrate and the enzyme being additionally activated by another Mg^{2+} [4].

4. Additional effects on enzyme proteins may be:
Mg^{2+} causes conformational changes of enzymes during the catalytic process, e. g. Na^+-K^+-transporting Na^+, K^+-ATPase [66] and Ca^{2+} pumping sarcoplasmic reticulum ATPase [118].
Mg^{2+} can be necessary for binding of subunits to form the active multienzyme-complex e. g. F_1-ATPase [1].
Mg^{2+} (0.4 mmol/l) activates aldehyde dehydrogenase by dissociating the tetrameric state with only two active subunits per tetramer to the dimeric state with two active subunits per dimer [140].
The inhibition is caused by (unspecific) binding of Mg^{2+} to the enzyme (especially to tyrosyl residues) at higher Mg^{2+} concentration, as known from binding of Mg^{2+} to serum albumin. Furthermore, Mg^{2+} at higher concentration competes with MgS at the active centre [4] or enzymatically inactive higher Mg-substrate complexes may be formed.

Thermodynamic effects of Mg^{2+}

When, in an enzyme-catalyzed reaction $A + B \overset{E}{\rightleftarrows} C + D$ [E may be an Mg^{2+}-activated enzyme or not], the substrates (A, B) or products (C, D) form Mg-chelates with different stability, and when the Mg-chelates and the noncomplexed substances have a different affinity to the enzyme, the apparent equilibrium constant K' changes with the Mg^{2+} concentration, according to the following formula

$$K' = K \frac{(1 + K_C[Mg^+]) \cdot (1 + K_D[Mg^{2+}])}{(1 + K_A[Mg^{2+}]) \cdot (1 + K_B[Mg^{2+}])}$$

K_A, K_B, K_C and K_D are the Mg^{2+} complex-forming constants of A, B, C and D. In Fig. 2, an example is demonstrated.

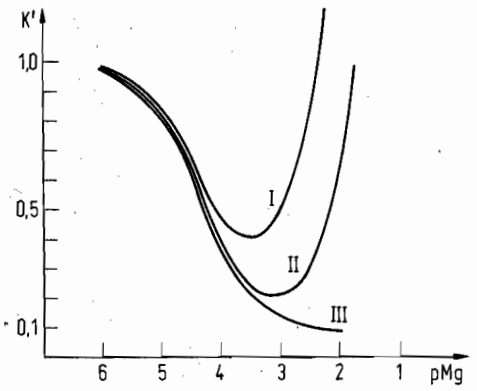

Fig. 2: pMg dependency of the equilibrium constants K'. For calculation see text. To enable a better comparison of K' for different enzymes, K has been assigned to a value of 1.0. I adenylate kinase, II pyruvate kinase, III phosphofructokinase. From [52].

Reaction of Mg^{2+} with cell structures

Phospholipids: Mg^{2+} and Ca^{2+} form complexes with phospholipids that are integral parts of the various cell membranes (endoplasmic reticulum, sarcoplasmic reticulum, mitochondria, plasma membranes). It can be assumed that with regard to Mg^{2+} and Ca^{2+} concentrations in the intra- and extracellular spaces, most of the phosphate groups in phospholipids are combined with Mg^{2+} and Ca^{2+}.
On the cytosolic side of the plasma membrane, not only Mg^{2+} but also polyamines could be bound. The situation is more complicated because the various phospholipids are not homogeneously distributed in the membrane [123] and various phospholipids have different affinities to Mg^{2+} and Ca^{2+}. However, the reported Ca^{2+} binding constants for the same phospholipids range from 10^5 to 10^1 $(mol/l)^{-1}$ [69, 102, 129]. Ca^{2+} has a stronger and more specific effect on phospholipids than Mg^{2+} [108]. Moreover, Ca^{2+}

binding to cell membranes is enhanced by various hormones [glucocorticoids, insulin, glucagon, adrenalin [76, 95]]. Others have found a reduced Ca^{2+} binding by insulin [130]. The binding of Ca^{2+} is inhibited by Mg^{2+} [130]. With endoplasmic reticulum from adipocytes the various Ca^{2+} binding sites were inhibited noncompetitively by Mg^{2+} [19]. Binding of divalent cations to phospholipids decreases their mobility within the membrane, thus decreasing membrane fluidity and inducing structural changes and electrostatic effects [84]. Binding of Mg^{2+} to the surface of Ehrlich ascites tumor cells decreased their surface potential (zeta-potential) [125]. As a consequence of these effects, membrane permeability decreases. In addition, the activity of membrane-bound, phospholipid-dependent enzymes as well as membrane-receptor-activated enzymes may be changed [9].

Interestingly, in E. coli grown in Mg-deficient media, membrane permeability increased and the composition of phospholipids in the cell membrane changed [61]. This defect could not be normalized by addition of Mg^{2+} alone but required protein synthesis [59].

Whether all these effects on phospholipids and cell membranes play a significant role in clinical or experimental Mg deficiency is not elucidated.

Nucleic acids: The binding of Mg^{2+} to nucleic acids by forming outer sphere complexes reduces the repellent action of negatively charged phosphate groups. A cross-linking of contiguous nucleic acid helices by Mg^{2+} is unlikely because of the relatively wide distances between two phosphate residues. However, any folding of a polynucleotide chain which brings two phosphate residues more closely together may result in the formation of Mg^{2+} inner sphere complexes. Moreover, the presence of Mg^{2+} will favour the folding of polynucleotide chains, e.g. tRNA [111]. Thus, one mole of phenyl-t-RNA binds four moles of Mg^{2+} and two moles of spermine. Their exact location within the tRNA molecule has been determined [106]. In rRNA and DNA the localisation and structural functions of Mg^{2+} are not defined. This also concerns the effects of Mg^{2+} on chromatin and on mitosis [73]. Ribosomal RNA binds up to 0.5 Mg^{2+}/phosphate, depending on ionic strength, polyamine and Mg^{2+} concentrations [47]. tRNA has a strong Mg^{2+} binding site ($K = 3 \cdot 10^4$ [mol/l]$^{-1}$) and weak binding sites with $K = 4 \cdot 10^2$ (mol/l)$^{-1}$ [136]. This explains why the formation of the initiation complex in protein synthesis also depends on magnesium ions [12]. After removal of Mg^{2+} by EDTA [146] or by growing of bacteria in Mg-deficient media [94], the ribosomes disintegrate and disappear. Thus, further growth and further reduction of intracellular Mg^{2+} ion concentration are stopped.

In protein biosynthesis, the formation of the initiation complex at the small ribosome subunit includes binding of tRNA to mRNA by pairing three bases. At high Mg^{2+} concentration, pairing of two bases is sufficient to bind some amino acyl-tRNAs. Therefore, to prevent miscoding [31, 105, 138] and to facilitate the action of initiation and dissociation factors [87, 88, 137] in protein biosynthesis, a sufficiently low and constant Mg^{2+} ion concentration is necessary.

To summarize, due to the above-mentioned effects on enzymes and cellular structures, Mg^{2+} is involved in most reactions of carbohydrate, lipid, nucleic acid and protein metabolism, as well as in energy-producing (glycolysis, oxidative phosphorylation) and energy-consuming reactions (active transport, muscle contraction).

Distribution of Mg^{2+}

All above-mentioned effects of Mg^{2+} change with the concentration of free Mg^{2+} ions, according to the law of mass action. Therefore, metabolic or regulatory effects of Mg^{2+} can only occur where the Mg^{2+} ion concentration in the various compartments changes.

Total Mg^{2+} concentration in human serum is 0.89 mmol/l [36]. Since part of it is bound to serum albumin and other substances in serum, the concentration of free extracellular Mg^{2+} ions is 0.5 mmol/l.

Cellular Mg^{2+} contents are 3—9 mmol/kg wet weight, depending on cell type [36]. Most of the Mg^{2+} is localized in the microsomes and mitochondria. In liver mitochondria about 40% of the Mg^{2+} are found in the intermembranous space and 50% in the matrix. The outer and inner membrane each contain only about 5% [15]. In another investigation [68] the distribution within the mitochondria was: intermembranal compartment: 36.5%, matrix: 28.5%, inner membrane: 14.3%, outer membrane: 4.3%. The high Mg^{2+} content in the intermembraneous compartment is caused by Mg^{2+} binding proteins. One protein with a molecular weight of 150 000 dalton binds 300 nmol Mg^{2+}/mg protein with a dissociation constant of 0.37 mmol/l. Another protein with a molecular weight of 100 000 dalton binds 20 nmol/mg protein with a dissociation constant of 1.0 µmol/l [16]. The biological function of these Mg^{2+} binding proteins is not elucidated.

The high Mg^{2+} content in the mitochondrial matrix is caused by the relative high amount of matrix volume (approx. 55% [85]) and the high ATP content of the matrix. It represents probably no enrichment of free Mg^{2+} because there is a similar concentration of free Mg^{2+} in mitochondria and cytosol (Tab. 1).

The high Mg^{2+} content of the microsomes [45] [deriving from the endoplasmic reticulum] may result from binding of Mg^{2+} to adhering ribosomes [from rough endoplasmic reticulum].

The concentration of free Mg^{2+} ions in the cytosol is about 1 mmol/l (pMg = 3) as has been determined by various authors using various methods (Tab. 1).

Mg^{2+} is not equally distributed in the cytosol because of enrichment of Mg^{2+} at negatively charged membranes and at the surface of polyanions. Moreover, transport and exchange of Mg^{2+}, Ca^{2+} and other ions

Tab. 1: Intracellular Mg^{2+} ion concentration in various cell types.

cell type	species	$[MG^{2+}]ICF$ mmol/l	remarks	method	Ref.
red blood cells	human	0.25	aerobic	^{31}P NMR	63
		0.67	anaerobic	^{31}P NMR	63
		0.45	aerobic	cation exchange	2
		0.57	anaerobic	cation exchange	2
		0.5		glycolysis	50
		0.4		A 23187	41
brain	rat	1		Mg binding site	142
		1.5		aconitate hydratase	142
kidney	rat	2.2		Mg binding site	142
		1.0	activity*	electrode	55
liver	rat	1.5		Mg binding site	142
		1		aconitate hydratase	142
		0.9	activity*	electrode	55
		1.6		enzymatic	50
liver mitochondria	rat	1.6		enzymatic	50
muscle	rat	1	activity*	electrode	55
		2.8		enzymatic	50
	frog	0.6		^{31}P NMR	64
		4.4		^{31}P NMR	25
Ehrlich ascites tumor	mouse	0.44		^{31}P NMR	65
		1.6		enzymatic	50
		0.8	activity*	electrode	55

* values expressed as activity [a], because of the unknown activity coefficient [γ] in the intracellular fluid.
[$a = \gamma \cdot c$] γ = activity coefficient, c = concentration, $\gamma \geqslant 0.5$]

take place in mitochondria, endoplasmic and sarcoplasmic reticulum.
Since the transfer of ADP and ATP across the mitochondrial membrane occurs with the uncomplexed nucleotides [80], the Mg^{2+} ion concentration in the cytosol or mitochondria may be changed when in transient states a rapid translocation and alteration in the concentration of nucleotides has taken place.
Under steady state conditions in the various intracellular compartments, free Mg^{2+} and bound Mg^{2+} is in equilibrium, because $^{28}Mg^{2+}$ is almost completely exchanged. In the heart, 98% of the Mg^{2+} is exchanged at a uniform rate constant [107]. There are rapidly (liver, kidney, heart) and slowly (erythrocytes, muscle, brain) $^{28}Mg^{2+}$-exchanging organs and cells [5, 119, 120]. The reason for this behaviour is unclear.
Such a cellular system, with free Mg^{2+} and bound Mg^{2+} being in an exchanging equilibrium, represents an Mg^{2+} buffer. The pMg of the intracellular Mg^{2+} buffer [49, 142] as shown above by the intracellular Mg^{2+} ion concentration is in the range of 3. At this Mg^{2+} concentration the Mg^{2+} dependent enzymes are optimally or almost optimally activated by Mg^{2+} [48]. The low intracellular Mg^{2+} concentration is important to prevent miscoding [31, 105, 108] and to facilitate the action of initiation and dissociation factors [87, 88, 137] in protein biosynthesis and to involve the actions of polyamines [141].

Cellular Mg^{2+} transport

The uptake studies with $^{28}Mg^{2+}$ have shown that Mg^{2+} is transported across cell membranes. In rat heart, KB cells and E. coli cells, the transport of $^{28}Mg^{2+}$ was found to obey Michaelis-Menten kinetics. The K_m-values for heart, KB and E. coli cells were 0.57 mmol/l [107], 0.1 mmol/l [11] and about 0.01 mmol/l [100, 132]. In all these cells, influx and efflux of Mg^{2+} seem to be coupled and to be mediated by an energy-dependent carrier system. Since Mg^{2+} transport of E. coli is genetically determined [101, 109] it may be assumed that Mg^{2+} transport is facilitated or controlled by specific proteins. Since isoproterenol inhibited $^{28}Mg^{2+}$ uptake in lymphoma cells [90], it is possible that the β-adrenergic receptor is involved in the influx mechanism of $^{28}Mg^{2+}$ in animal cells. However, in these experiments flux rates under steady state conditions of Mg^{2+} distribution and not net transport rates of Mg^{2+} were measured.
An active net uptake of Mg^{2+} occurs when e.g. E. coli cells are growing in a medium with low Mg^{2+} concentration (10^{-6}—10^{-7} mol/l) [57], because the intracellular concentration of free Mg^{2+} in E. coli is also in the millimolar range [57].
Cold-stored [1 °C] liver slices lost about 10% of their Mg^{2+} and after reincubation at 38 °C reaccumulated Mg^{2+}, indicating some energy dependent net Mg^{2+} uptake [122].
Since the membrane potential in animal cells is up to 60—90 mV, inside negative, the intracellular (Mg^{2+}) should be 10^2—10^3 times higher than the extracellular (Mg^{2+}), if Mg^{2+} is transported by an electrophoretic uniporter. However, the intracellular (Mg^{2+}) amounts only to 1—1.5 mmol/l compared to an extracellular (Mg^{2+}) of 0.5 mmol/l. Therefore one should conclude that in cells with high membrane potential there should be (a carrier-mediated) diffusion into the cell and an active transport of Mg^{2+} out of the cell. If Mg^{2+} is transported into the cell by antiport or symport, Mg^{2+} transport and distribution should be independent of membrane potential. The reduction of $^{28}Mg^{2+}$ uptake in KB cells by ouabain [11] indicates that there may be one component of $^{28}Mg^{2+}$ uptake coupled to active Na^+ transport out of the cell (antiport).
These relationships should be taken into account, when the behaviour of the intracellular Mg^{2+} content in Mg deficient or pathological states or after Mg supplementation is considered.

Mitochondrial Mg^{2+} transport

The experiments on Mg^{2+} uptake into mitochondria [17] can be reinterpreted as Mg^{2+} transport being energized by a potential or pH gradient according to Mitchell's chemi osmotic theory. Thus heart mitochondria accumulate large amounts of Mg^{2+} and release H^+ in presence of anorganic PO_4. A net efflux of Mg^{2+} from liver mitochondria is found when the mitochondria take up Ca^{2+} [131]. Heart mitochondria take up

more Mg^{2+} than liver mitochondria [17, 74]. The Mg^{2+} influx as measured with $^{28}Mg^{2+}$ also exhibits Michaelis-Menten kinetics with a K_m-value of 0.7 mmol/l. $^{28}Mg^{2+}$ influx competes with K^+ influx and vice versa [35]. Therefore it seems possible that Mg^{2+} and K^+ are transported by the same carrier mechanism that is different from Ca^{2+} transport [35] and unrelated to adenine nucleotide translocation [35, 80]. Since Mg^{2+} efflux also is inhibited by cyanide [35], some coupling of Mg^{2+} influx and Mg^{2+} efflux in mitochondria is possible. The energy-dependent slow net transport of Mg^{2+} across the inner mitochondrial membrane into the matrix probably produces no (Mg^{2+}) gradient, because mitochondrial and cytosolic Mg^{2+} concentrations are similar (Tab. 1). Since the membrane potential of the mitochondrial membrane amounts to 180 mV, inside negative, the situation is thermodynamically similar as discussed for the cell membrane.

Microsomal Mg^{2+} transport

Ca^{2+} transport into the endoplasmic and sarcoplasmic reticulum is coupled to hydrolysis of ATP by Ca^{2+} ATPase. During this process Mg^{2+} may be secondarily transported for charge compensation. A relation of 2 Ca^{2+} for 1 Mg^{2+} and 2 K^+ was suggested. However, charge compensation for primarily actively transported Ca^{2+} occurs also by readily permeating anions e.g. phosphate (or oxalate). For review see [22].

Alteration of Mg^{2+} distribution

The Mg^{2+} distribution may be changed by:

I. Elevation of the extracellular Mg^{2+} concentration
As shown with ascites tumor cells [48] and E. coli [57], elevation of extracellular Mg^{2+} concentration leads to an increased binding of Mg^{2+} to the cell membrane. At these conditions there is only a small increase in the intracellular Mg^{2+} ion concentration, if at all. Since there is an intracellular Mg^{2+} buffer [49, 142] the Mg^{2+} taken up is buffered. Moreover, the Mg^{2+}-dependent reactions are dependent on log (Mg^{2+}) and since the intracellular pMg is in the range of 3 (the pMg optimum of the Mg-dependent enzymes), an alteration of the intracellular Mg^{2+} concentration would have no significant effect on the activity of Mg^{2+}-dependent enzymes. Analogous considerations would hold for a reduction of the extracellular Mg^{2+} concentration.
This behaviour is supported by the properties of the Mg^{2+} transport system, representing a saturable coupled influx and efflux of Mg^{2+}. Such a mechanism would not cause a net increase or decrease of intracellular Mg^{2+} concentration at increased or decreased extracellular Mg^{2+} concentrations.
Therefore, one can conclude that mainly extracellular effects are produced along membranes by changing the extracellular Mg^{2+} concentration and particularly by changing the competition of Mg^{2+} with Ca^{2+}.

II. Mg deficiency
Reciprocally, a decrease in intracellular Mg^{2+} content can be produced when cells are grown at reduced extracellular Mg^{2+} concentrations, for example when young rats are fed a Mg-deficient diet or cells are grown in media with low Mg^{2+} concentration.

Metabolic alterations in Mg deficiency

The main metabolic effects under these experimental conditions are: The serum Mg^{2+} concentration and the serum protein concentration, especially that of γ-globulins, is decreased in Mg-deficient animals [6, 37, 117, 128]. In cell cultures with low-Mg^{2+} media, the biosynthesis of protein, DNA and RNA is reduced in a decreasing order indicated by their sequence [53, 124].
Mineral metabolism: In Mg deficiency, the cellular amounts of Na^+, Ca^{2+} (and in some cell types cAMP [62, 116]) are increased and the cellular amounts of K^+ and Mg^{2+} are decreased. Amounts of Na^+, K^+, Mg^{2+} and Ca^{2+} reported by various authors in various tissues are summarized in Table 2.

Tab. 2: Relative Changes in Electrolyte Contents of Total Organs in Mg Deficiency in % (Controls = 100%)

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Ref.
Muscle	112	106	72	143	45
	124	90	92	—	38
	134	89	86	122	147
	156	89	87	132	89
	155	87	87	—	51
	157	83	95	212	93
Heart	112	87	96	136	45
	108	95	85	—	51
	120	82	91	362	93
Liver	106	80	74	158	45
	99	102	103	—	38
	—	103	99	96	89
	119	101	95	—	51
	126	88	93	214	93
Kidney	107	94	100	230	45
	—	102	95	423	89
	100	102	94	—	51
	103	102	98	525	93
Fetuses	108	63	78	—	56
Tumor cells in vitro	195	45	51	227	53

The effects of Mg deficiency are greater in muscle and heart and smaller in liver and kidney. The effects are greater in more rapidly growing animals or cells.

Tab. 3: Changes of intracellular Na^+, K^+ and Mg^{2+} content and extracellular and intracellular fluid volume in Mg deficiency. In brackets: relative changes of intracellular Mg^{2+} in %.

	$\Delta[Na^+]_{ICF}$	$\Delta[K^+]_{ICF}$	$\Delta[Mg^{2+}]^t_{ICF}$	ΔECF	ΔICF	calculated from Ref.
	mmol/l		mmol/kg	ml/kg wet wt.		
muscle	+ 1.7	− 9	−0.45 [2.7]	+ 36	−36	38
	− 2.0	−26	−2.1 [17.8]	+ 53	−42	147
	− 3.7	−14	−2.5 [21.0]	+121	−95	51
liver	+ 6.9	− 5	−0.4 [2.4]	− 33	+56	38
	+25.5	−42	+0.1 [1.0]	− 74	+89	51

In Tab. 3 the changes in extracellular, intracellular fluid volume and intracellular Na^+, K^+ and Mg^{2+} contents are listed. The values show an increase in intracellular (Na^+) and a decrease in intracellular (K^+) and a decrease in total intracellular Mg^{2+}. The alteration in the Ca^{2+} distribution between intra- und extracellular space was not analysed because of the high extra-intracellular (Ca^{2+}) gradient, the strong mitochondrial and endoplasmic enrichment and the poor accuracy of the ECF determination.

The values in Tab. 3 are rather inaccurate because of the error in the determination of the extracellular fluid volume and because different substances ($^{36}Cl^-$ [38, 147], sucrose [51]) were used. The increase in ECF in muscle in Mg deficiency comes from the enlargement of the transverse tubule system of the sarcoplasmic reticulum, being in contact with the extracellular fluid [96]. The Mg deficiency-induced change in intracellular Na^+ and K^+ concentration is produced by the increase of cell permeability as found by flux experiments [8, 53, 125] and with red cell ghosts [121].

The elevated intracellular (Na^+) should enhance Na^+, K^+-ATPase activity. After incubation in vitro there was the same Na^+, K^+-ATPase activity in membrane preparations of normal and Mg-deficient Yoshida ascites tumor cells [53]. Therefore, to test Na^+,K^+-ATPase activity in intact cells, binding of (^3H) ouabain was measured. In Mg deficient Yoshida ascites tumor cells a small increase in (^3H) ouabain binding was found [8], whereas in Mg deficient tumor cells $^{22}Na^+$ efflux was enhanced by about 30% [8, 125]. From these results it seems possible that the increased $^{22}Na^+$ efflux in Mg deficient tumor cells may in part be caused by an increased Na^+, K^+ cotransport or Na^+-Na^+ exchange. However, as yet there is no exact analysis of Na^+ and K^+ flux components in Mg-deficient cells.

The increase in cellular Ca^{2+} content may be due to an increased binding of Ca^{2+} to the cell membrane and an increased intracellular uptake of Ca^{2+}. The intracellular distribution of this additionally accumulated Ca^{2+} is unknown, especially the resulting cytosolic concentration of free Ca^{2+} ions.

Part of the Ca^{2+} taken up in Mg deficiency may be stored in the intracellular Ca^{2+} stores. In electronmicroscopic pictures of the left papillary muscle of Mg-deficient dogs, we found electron-dense particles, probably Ca phosphate, in the sarcoplasmic reticulum. Such a redistribution of cellular Ca^{2+} was also found with isolated hepatocytes and may be a general property of intracellular Ca^{2+} metabolism. Addition of 10^{-6} mol/l noradrenalin to intact cells by an α-adrenergic mechanism caused a 40% reduction of mitochondrial Ca^{2+} content, a rise of cytosolic free Ca^{2+} from 0.10 to 0.45 µmol/l, an increased Ca^{2+} content of the microsomal fraction (2.7 times) and an increased Ca^{2+} efflux out of the cell [99].

The cytosolic Ca^{2+} concentration is known to have important regulatory effects on various enzymes, e.g. adenylate cyclase (from brain), phosphodiesterase, Ca^{2+} ATPase, protein kinase(s) via calmodulin, on membrane-bound guanylate cyclase, on various endocrine and exocrine functions, transmitter release, heart and muscle contraction and growth of cell cultures. For reviews see [22, 115].

Since the decrease in the cellular Mg^{2+} content in Mg deficiency is rather small and since intracellular Mg^{2+} is buffered and in the range of the pM optima (see above) it is unlikely that the metabolic alterations in Mg deficiency are brought about by a decrease in the intracellular Mg^{2+} concentration. Since protein biosynthesis is reduced at reduced intracellular K^+ concentration [21] or at increased Cl^- [145] or Ca^{2+} [77] concentration, the metabolic effects in severe Mg deficiency may be caused by the increased permeability and by an alteration of electrolytes other than Mg^{2+}.

To prove this assumption we have increased the permeability of Yoshida ascites tumor cells in vitro by addition of the ionophore X-537 A and compared the effects in Mg-deficient and X-537 A-treated Yoshida cells.

Treatment of Yoshida cells with X-537 A produced an increase in intracellular (Na^+), (Ca^{2+}) and (cAMP) and a decrease in intracellular (K^+) as was the case in Mg deficiency. The Mg^{2+} content remained constant [54].

(An increase in cAMP by Mg deficiency [62, 116] and by X-537 A [127] was also found in adipocytes and by the Ca^{2+} ionophore A 23187 in pancreatic islets [78] and macrophages [44]. In the latter case the increase in (cAMP) was mediated by prostaglandins. In other cells Ca^{2+}-ionophores induce a decrease in (cAMP) and an increase in (cGMP) [22]. A simultaneous reversible increase of intracellular [Ca^{2+}] and (cAMP) was also observed in hepatointoxication with thioacetamide and in liver tumors induced by 4-dimethylaminoazobenzene [7].)

Furthermore, in the presence of X-537 A the intracellular electrolyte concentrations can be manipulated due to their extracellular concentrations [54].

In these experiments we found the same inhibition in the biosynthesis of protein, DNA and RNA (decreasing in this order) in Mg deficient as well as in X-537 A-treated cells. The same inhibition of DNA, RNA and protein biosynthesis was also produced by increa-

sing cellular Ca^{2+} only [54]. From these results, we concluded that the metabolic effects in (severe) Mg deficiency were caused by an increase in cell permeability and thus by the altered intracellular Ca^{2+} and/or K^+ concentrations. In similar in vitro-experiments with the parotid gland, adrenalin or A 23187 inhibited protein biosynthesis by a rise in intracellular free (Ca^{2+}) via an α-adrenergic mechanism [77].

Secondarily, in Mg deficiency, an alteration in some cellular proteins or enzymes might be involved because there was no normalization in Mg-deficient E. coli and Yoshida ascites tumor cells when Mg^{2+} had been added, but only after growth and protein synthesis had taken place [53, 59]. This agrees with the result that the activity (concentration) of elongation factor 1 and 2 was reduced in lymphocytes from Mg-deficient rats [42]. These effects were not produced by an insufficient activation of Mg^{2+} dependent enzymes because sufficient Mg^{2+} was added in the tests. It is unclear why some proteins or enzymes are more reduced than others.

Energy metabolism in Mg deficiency: In Mg-deficient Yoshida ascites tumor cells grown in vitro in a Mg-deficient medium, glucose consumption and lactate formation are increased whereas ATP content was not changed [53]. In Mg depleted Ehrlich ascites tumor cells, respiration and glycolysis were increased [126]. The mechanism may be the increased permeability of the cell membrane and thus an increased ATP-consuming transport of Na^+ and K^+ [8, 53, 125]. Thus energy metabolism in Mg deficiency would be regulated by the phosphate potential (ATP)/(ADP) × (P_i) as is usually the case.

Moreover, glycolysis may be increased by the increased (cAMP), activating phosphofructokinase. The cytosolic (Ca^{2+}), increased in Mg deficiency, may activate phosphorylase kinase, leading to increased glycogenolysis and glycolysis, stimulation of pyruvate dehydrogenase complex by activation of pyruvate dehydrogenase phosphatase, stimulation of tissue lipases, activation of the glycerophosphate shuttle for NADH and stimulation of the mitochondrial oxidation of β-hydroxybutyrate. All these reactions will normalize a reduced (ATP)/(ADP) × (P_i) ratio, and may lead to a more reduced state of the mitochondrial pyridine nucleotides and a higher mitochondrial (Ca^{2+}) [83]. The mechanism by which the redox state of the mitochondrial pyridine nucleotides controls mitochondrial (Ca^{2+}) is not known.

In liver slices from Mg deficient rats, oxygen uptake was lower than in the controls [46]. This effect is probably due to a decrease in the number of mitochondria and not to a defect of the single mitochondria because oxygen uptake per mg protein was identical in isolated mitochondria from Mg deficient and control rats. However, no ATP levels were measured in these experiments. The effect of Mg-deficiency on oxidative phosphorylation is controversial. In Mg deficient E. coli a partial uncoupling was found [60]. In mitochondria from Mg deficient animals no uncoupling [13], or a reduction of the P:O ratio was reported [34, 46, 143]. The latter effect may be in agreement with the swelling of mitochondria usually seen in electron microscopic pictures from Mg deficient cells [96].

The relatively small reduction of the P : O ratio in isolated mitochondria from Mg deficient animals may be due to lysosomes, which are known to reduce ATP synthetase activity in mitochondria [82] or may be due to an increased content of free fatty acids or to an altered electrolyte metabolism of the mitochondria in Mg deficiency. Since oxidative phosphorylation according to Mitchell's chemiosmotic theory is coupled to the potential and pH gradient of the mitochondrial membrane, some essential characteristics of the mitochondrial Ca^{2+} metabolism are considered because this is a very active function, depending on mitochondrial membrane potential.

The elevated intracellular Na^+ concentration in Mg deficiency can induce a rapid (within a few minutes) release of stored Ca^{2+} from the mitochondria by an exchange (antiport) of 3 Na^+ for 1 Ca^{2+} [27]. In recent experiments with heart mitochondria an exchange of 2 Na^+ per 1 Ca^{2+} was found [3]. In the latter case, this process would not generate an electrical current and would be independent of the mitochondrial membrane potential. The involved Na^+-Ca^{2+} antiporter was partially purified and incorporated in phospholipid vesicles [97]. Moreover, the Na^+-Ca^{2+} antiport in heart mitochondria is activated by (K^+) ($K_m = 17-19$ mmol/l [29]) and (in brain mitochondria) is inhibited by Mg^{2+} [24]. The activation by K^+ and the inhibition by Mg^{2+} has no regulatory effect under intracellular conditions, because cytosolic (K^+) is far above K_m and cytosolic (Mg^{2+}) remains rather constant. The less affected mitochondria from liver, lung etc. may possess an additional separate efflux carrier which ejects Ca^{2+} against its electrochemical gradient independent of (Na^+). For review see [104]. The kinetic parameters and the quantitative expression in various tissues are listed in Tab. 4. From the K_m-values for Na^+ one can conclude that this effect plays a role in experimental Mg deficiency and that some tissues are affected to a different extent.

Tab. 4: Half maximal concentration (K_m in mmol/l) and maximal velocity (V_{max} in nmol Ca^{2+}/mg protein × min) of Na^+-induced Ca^{2+} release from various mitochondria

Tissue	K_m	V_{max}	Ref.
Brain	9	12	28
	—	16	67
Heart	8	14	27
	—	10	67
Adrenal cortex	12	18	28
Kidney medulla	—	11	67
Leg muscle	7	5	28
Masseter muscle	8	4	28
Parotid gland	8	5	28
Liver	—	1.6	67
Lung	—	2.4	67

The released Ca^{2+} is taken up again by the mitochondria by an energy-requiring transport consisting of an electrophoretic uniport, depending on the mitochondrial membrane potential [86]. This Ca^{2+} uptake is higher in liver than in heart mitochondria [71]. The probable Ca^{2+} transporting protein (calciphorin, molecular weight 3000) was isolated from the inner mitochondrial membrane [72]. An increased energy-consuming recycling of Ca^{2+} in mitochondria from Mg deficient cells results in an uncoupling of oxidative phosphorylation. Ca^{2+} uptake in mitochondria is inhibited by Mg^{2+} [71, 103, 134]. The action of Mg^{2+} is combined with a change in the kinetics of Ca^{2+} uptake from hyperbolic to sigmoidal [30]. Thus, in vitro (Mg^{2+}) can influence cytosolic steady state (Ca^{2+}) [103]. However, when the cytosolic (Mg^{2+}) remains constant, this effect has no regulatory function.

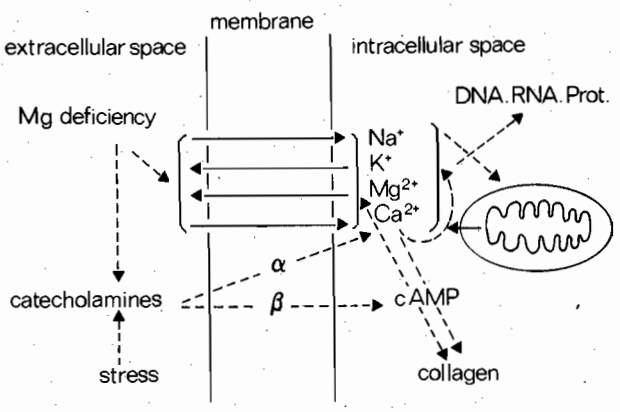

Fig. 3: Biochemical mechanisms under Mg deficiency and stress (schematic representation)

Superimposed effects in Mg deficiency

cAMP may increase cell membrane permeability for some ions [43] probably by activating protein kinase which phosphorylates a membrane-bound protein [81, 115]. We, therefore, charaterized its role in Mg deficiency. As a target cell the rat heart was chosen because the alterations in electrolyte [51] and cAMP [62] content induced by Mg deficiency were highest in this organ. Since the cAMP content may be increased by catecholamines and stress, young rats were fed with Mg deficient diets of varying Mg^{2+} content for 16 weeks. During the last 12 weeks of this period the rats were stressed by noise of varying degrees [58]. At the end of the experiment, we measured the Mg^{2+} concentration in the serum, Na^+, K^+, Ca^{2+}, Mg^{2+} and hydroxyproline content in the heart, and adrenalin and noradrenalin excretion in the urine.

In Mg deficiency, with decreasing Mg^{2+} concentration in the serum, there was a strong increase in noradrenalin excretion and a small increase in adrenalin excretion.

The decrease in K^+ and Mg^{2+}content as well as the increases in Na^+, Ca^{2+} and hydroxyproline content in the hearts were found to be correlated to noradrenalin excretion. From these results one can conclude that catecholamines are involved and may multiply the effect of Mg deficiency.

The increase in hydroxyproline had been due to an increase in collagen content without any necrosis as was additionally demonstrated by electron microscopy. The increase in collagen content is caused by a stimulation of fibrocytes as can be seen by the increase in their endoplasmic reticulum. The stimulation may be caused by cAMP [92] and/or by Ca^{2+} [33]. However, the effects of cAMP and Ca^{2+} on collagen biosynthesis involve activation and inhibition [10, 33] depending on their concentrations. Also the type of the newly synthesized collagen may be changed by cAMP and Ca^{2+} [32].

The biochemical mechanisms of Mg deficiency and catecholamines are summarized in Fig. 3.

The increase in cell membrane permeability produces a decrease in cytosolic (K^+) and possibly (Mg^{2+}) and an increase in cytosolic (Na^+) and (Ca^{2+}). The increased cytosolic (Na^+) induces a release of mitochondrial Ca^{2+} [27, 28, 67] and a further elevation of cytosolic (Ca^{2+}) and a secondary accumulation of Ca^{2+} in the endoplasmic reticulum. The increased cytosolic (Ca^{2+}) increases cell membrane permeability for intracellular K^+ in erythrocytes and some other cells [20, 39, 112] by binding of Ca^{2+} to a site at the inner surface ($K_D = 3-5 \cdot 10^{-7}$ mol/l) [112]. The increased cytosolic (Ca^{2+}) may increase (cAMP) in some cell types (via calmodulin-adenylate cyclase) that also enhances permeability. cAMP again may release some Ca^{2+} from the mitochondria [75] when palmityl CoA is the substrate.

At a reduced serum Mg^{2+} concentration, probably by reduced competition with extracellular Ca^{2+}, more catecholamines are released [58]. This effect is further stimulated by stress. The liberated catecholamines increase (cAMP) by a β-adrenergic effect. Also catecholamines act by an α-adrenergic effect. After binding to α-adrenergic receptors they increase $^{45}Ca^{2+}$ influx and Ca^{2k} efflux and mobilize mitochondrial Ca^{2+}. In liver cells these effects result in an increase of the cytosolic (Ca^{2+}) from 0.10 to 0.45 µmol/l [99] and in a net efflux of K^+ [20, 39] and Ca^{2+} [14, 20, 23, 39, 99]. In other cells α-agonists cause a net influx of Ca^{2+} [39]. There are evidences that α-agonists act by activation of protein kinase by Ca^{2+} and phosphorylation of some proteins in a similar manner to cAMP [39]. Both α- and β-adrenergic mechanisms multiply the effect of Mg deficiency. Moreover, there may be a catecholamine (β-receptor)-induced inhibition of Mg^{2+} influx at a specific Mg^{2+} channel [135].

The situation is further complicated since the concentration of catecholamines in the ECF is not constant with time because of reuptake, oxidation, methylation and excretion of catecholamines. Also, there may be a specific subsensitization to the effects of catecholamines. After application of catecholamines the number of

β-receptors [98] or the efficiency of coupling to adenylate cyclase [133] was reduced.
Finally, the altered cytosolic (K^+) and/or (Ca^{2+}) may inhibit the synthesis of protein, DNA and RNA. Increased (cAMP) and/or (Ca^{2+}) may stimulate collagen synthesis. Energy metabolism may be stimulated by the increased ATP-consuming Na^+-K^+-transport and the increased (cAMP) and (Ca^{2+}) as described above.
These mechanisms may be different for various cell types because there are differences in cell permeability, pump activity for Na^+, K^+ and Ca^{2+}, mitochondrial Ca^{2+} release, number and affinity of catecholamines (α, β) receptors, activity and Ca^{2+} sensitivity of adenylate cyclase, guanylate cyclase and phosphodiesterases.

The effects of Ca^{2+} as shown with brain adenylate cyclase and phosphodiesterase depend on (Ca^{2+}). The enzymes exhibit different pCa optima [113]. Besides its action on calmodulin, Ca^{2+} is responsible for the stimulation of adenylate cyclase by GTP [70]. In other tissues an increase in (Ca^{2+}) inhibits adenylate cyclase and activates membrane-bound guanylate cyclase [144] increasing (cGMP) and decreasing (cAMP). By another regulatory mechanism cAMP activates a protein kinase in sarcoplasmic reticulum which phosphorylates a 22,000-dalton protein (phospholamban). Phospholamban augments Ca^{2+}ATPase and Ca^{2+} transport into the sarcoplasmic reticulum [139] in order to regulate cytosolic (Ca^{2+}). For summary see Fig. 4.

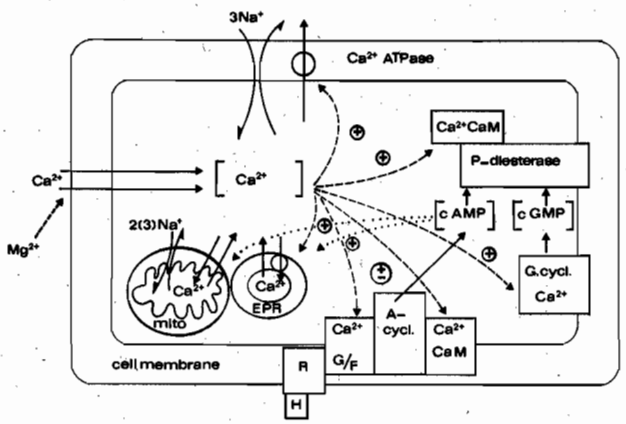

Fig. 4: Ca^{2+} metabolism and Ca^{2+}-cyclic nucleotide-interrelations (H, catecholamine, R, catecholamine receptor, G/F, GTP binding protein) Ca^{2+} leaks into the cell, partly inhibited by extracellular Mg^{2+}. Ca^{2+} is pumped out of the cell by Ca^{2+} ATPase and 3 Na^+ : 1 Ca^{2+}-exchange [110]
Cytolic Ca^{2+} is actively transported into the mitochondria by an electrophoretic uniporter. Ca^{2+} is released from mitochondria by 2 or 3 Na^+ : 1 Ca^{2+}-exchange or by an NAD/NADH dependent or cAMP dependent mechanism.
Cytosolic Ca^{2+} is actively transported into the endoplasmic or sarcoplasmic reticulum by Ca^{2+} ATPase and probably released by an increase in cytosolic (Ca^{2+}) or pH. The release mechanism is not elucidated.
Ca^{2+} activates Ca^{2+} ATPase, adenylate cyclase (brain) and membrane-bound phosphodiesterase via calmodulin (CaM) [79], Ca^{2+} is necessary for the activation of adenylate cyclase by GTP.

However, the effects of cAMP on Ca^{2+} metabolism are contradictory. In subcellular fractions of blood vessels it was reported that cAMP stimulates phosphorylation and Ca^{2+} uptake, that cAMP stimulates phosphorylation without an effect on Ca^{2+} uptake, that cAMP had no effect on phosphorylation and Ca^{2+} uptake and that cAMP stimulates Ca^{2+} uptake without an effect on phosphorylation. For review see [81].

Quantitative relations in Mg deficiency
The various transport systems are self-regulated. Na^+-K^+ pump is activated by cytosolic (Na^+) and Ca^{2+} pump by cytosolic (Ca^{2+}). Small alterations in cytosolic (Na^+) and (Ca^{2+}) produced by Mg deficiency should be normalized by self-regulation. Therefore, an important biochemical and medical question is at which degree the effects of Mg deficiency may become significant. In rats fed diets with a varying Mg^{2+} content, we found, that in unstressed rats there was a threshold at 0.7 mmol/l Mg^{2+} in the serum [58]. The most pronounced effects, i. e. the increase in hydroxyproline and Ca^{2+} content in the heart and noradrenalin excretion, became significant when the Mg^{2+} concentration in the serum had dropped below this value.
Another system in which the quantitative relationship between hypomagnesemia and Mg deficient effect was demonstrated is the resorption rate in unstressed pregnant rats and mice (in preparation). Again there was a threshold of serum Mg^{2+} concentration at 0.7 mmol/l below that resorption rate and mineralization defects became significant.

Bibliographie

[1] *Abrams, A., Jensen, C., Morris, D. H.:* Biochem. Biophys. Res. Comm. 69, 804—811 (1976).
[2] *Achilles, W., Klinger, R., Scheidt, B., Frunder, H.:* Acta biol. med. germ. 37, 1161—1166 (1978).
[3] *Affolter, H., Carafoli, E.:* Biochem. Biophys. Res. Comm. 95, 193—196 (1980).
[4] *Ahlers, J., Günther, T.:* Z. Naturforsch. 30 c, 412—416 (1975).
[5] *Aikawa, J. K., Rhoades, E. L., Harms, D. R., Reardon, J. Z.:* Am. J. Physiol. 197, 99—101 (1969).
[6] *Alcock, N. W., Shils, M. E.:* Próc. Soc. Exp. Biol. Med. 145, 855—858 (1974).
[7] *Anghileri, L. J., Heidbreter, M.:* Experientia 34, 415—416 (1977).
[8] *Averdunk, R., Ostapowicz, B., Günther, T.:* Z. Klin. Chem. Klin. Biochem. 13, 361—366 (1975).
[9] *Bakardjeva, A., Galla, H. J., Helmreich, E. J. M.:* Hormones and cell regulation 3, 11—27 (1979).
[10] *Baum, B. J., Moss, J., Breul, S. D., Crystal, R. D.:* J. Biol. Chem. 253, 3391—3394 (1978).
[11] *Beauchamp, R. S., Silver, S., Hopkins, J. W.:* Biochim. Biophys. Acta 225, 71—76 (1971).
[12] *Beaudry, P., Begard, E., Douzou, P., Grunberg-Manago, M.:* Eur. J. Biochem. 92, 613—619 (1978).
[13] *Beechey, R. B., Alcock, N. W., Mac Intyre, I.:* Am. J. Physiol. 201, 1120—1122 (1961).
[14] *Blackmore, P. F., Debaye, J.-P., Strickland, W. G., Exton, J. H.:* FEBS Letters 100, 117—120 (1979).

[15] *Bogucka, K., Wojtczak, L.:* Biochem. Biophys. Res. Commun. 44, 1330—1337 (1971).
[16] —, —: Biochem. Biophys. Res. Comm. 71, 161—167 (1976).
[17] *Brierley, G., Murer, E., Bachmann, E., Green, D. E.:* J. Biol. Chem. 238, 3482—3489 (1963).
[18] *Brintzinger, H., Fallab, S.:* Helv. Chim. Acta 43, 43—58 (1960).
[19] *Bruns, D. E., Mc Donald, J. M., Garett, L.:* J. Biol. Chem. 252, 927—932 (1977).
[20] *Burgess, G. M., Claret, M., Jenkinson, D. H.:* Nature, 279, 544—546 (1979).
[21] *Cahn, F., Lubin, M.:* J. Biol. Chem. 253, 7798—7803 (1978).
[22] *Carafoli, E., Crompton, M.:* The regulation of intracellular calcium. in: Current Topics in Membranes and Transport. F. Bronner, A. Kleinzeller (ed.) Vol. X: 151—216, Acad. Press (1978).
[23] *Chen, J. L. J., Babcock, D. F., Lardy, H. A.:* Proc. Natl. Acad. Sci, 75, 2234—2238 (1978).
[24] *Clark, A. F., Roman, I. J.:* J. Biol. Chem. 255, 6556—6558 (1980).
[25] *Cohen, S. M., Burt, C. T.:* Proc. Nat. Acad. Sci, 74, 4271—4275 (1977).
[26] *Cohn, M.:* Biochemistry 2, 623—629 (1963).
[27] *Crompton, M., Capano, M., Carafoli, E.:* Eur. J. Biochem. 69, 453—462 (1976).
[28] *Crompton, M., Moser, R., Lüdi, H., Carafoli, E.:* Eur. J. Biochem. 82, 25—31 (1978).
[29] *Crompton, M., Heid, I., Carafoli, E.:* FEBS Letters 115, 257—259 (1980).
[30] *Crompton, M., Sigel, E., Salzmann, M., Carafoli, E.:* Eur. J. Biochem. 69, 429—434 (1976).
[31] *Davies, J., Gilbert, W., Gorini, L.:* Proc. Nat. Acad. Sci. USA 51, 883—889 (1964).
[32] *Deshmukh, K., Sawyer, B. D.:* Proc. Natl. Acad. Sci. 74, 3864—3868 (1977).
[33] *Dietrich, J. W., Duffield, R.:* Endocrinol. 105, 1168—1172 (1979).
[34] *DiGiorgo, J., Vitale, J. J., Hellerstein, E. E.:* Biochem. J., 82, 184—187 (1962).
[35] *Diwan, J. J., Dazé, M., Richardson, R., Aronson, D.:* Biochem. 18, 2590—2595 (1979).
[36] *Ebel, H., Günther, T.:* J. Clin. Chem. Clin. Biochem. 18, 257—270 (1980).
[37] *Elin, R. J.:* Proc. Soc. Exp. Biol. Med. 148, 620—624 (1975).
[38] *Elin, R. J., Armstrong, W. D., Singer, L.:* Am. J. Physiol 220, 543—548 (1971).
[39] *Exton, J. H.:* Am. J. Physiol. 238, E2—E12 (1980).
[40] *Faller, L. D., Baroudy, B. M., Johnson, A. M., Ewall, R. X.:* Biochemistry 16, 3864—3869 (1977).
[41] *Flatman, P., Lew, V. L.:* Nature 267, 360—362 (1977).
[42] *Freude, K. A., Zieve, J. F., Zieve, L.:* J. Nutrit. 108, 1635—1641 (1978).
[43] *Friedmann, N., Park, C. R.:* Proc. Nat. Acad. Sci, USA 61, 504—508 (1968).
[44] *Gemsa, D., Seitz, M., Kramer, W., Grimm, W., Till, G., Resch, K.:* Exptl. Cell Res. 118, 55—62 (1978).
[45] *George, G. A., Heaton, F. W.:* Biochem. J. 152, 609—615 (1975).
[46] *George, G. A., Heaton, W. F.:* Int. J. Biochem. 9, 421—425 (1978).
[47] *Goldberg, A.:* J. Mol. Biol. 15, 663—673 (1966).
[48] *Günther, T.:* Habilitationsschrift, FU Berlin (1965).
[49] —: Z. Naturforsch. 21b, 1174—1177 (1966).
[50] —: Z. Naturforsch. 22b, 149—154 (1967).
[51] —: Z. Klin. Chem. Klin. Biochem. 8, 65—68 (1970).
[52] —: J. Clin. Chem. Clin. Biochem. 15, 433—438 (1977).
[53] *Günther, T., Averdunk, R.:* Z. Klin. Chem. Klin. Biochem. 8, 621—625 (1970).
[54] —, —: J. Clin. Chem. Clin. Biochem. 14, 365—371 (1976).
[55] *Günther, T., Dorn, F.:* Z. Naturforsch. 26b, 176—177 (1971).
[56] *Günther, T., Dorn, F., Merker, H. J.:* Z. Klin. Chem. Klin. Biochem. 11, 87—92 (1973).
[57] *Günther, T., Hoffmann, C. F.:* Z. Klin. Chem. Klin. Biochem. 11, 237—242 (1973).
[58] *Günther, T., Ising, H., Merker, H. J.:* J. Clin. Chem. Clin. Biochem. 16, 293—297 (1978).
[59] *Günther, T., Mariß, P.:* Z. Naturforsch. 23b, 334—338 (1968).
[60] —, —: Hoppe Seyler's Z. Physiol. Chem. 349, 623—631 (1968).
[61] *Günther, T., Richter, L., Schmalbeck, J.:* J. Gen. Microbiol. 86, 191—193 (1975).
[62] *Günther, T., Schmalbeck, J., Merker, H. J.:* Z. Klin. Chem. Klin. Biochem. 11, 233—236 (1973).
[63] *Gupta, R. K., Benovic, J. L., Rose, Z. B.:* J. Biol. Chem., 254, 6172—6176 (1978).
[64] *Gupta, R. K., Moore, R. D.:* J. Biol. Chem., 255, 3987—3993 (1980).
[65] *Gupta, R. K., Yushok, W. D.:* Proc. Nat. Acad. Sci. 77, 2487—2491 (1980).
[66] *Gupte, S. S., Lane, L. K.:* J. Biol. Chem. 254, 10362—10367 (1979).
[67] *Haworth, R. A., Hunter, D. R., Berkoff, H. A.:* FEBS Letters 110, 216—218 (1980).
[68] *Heaton, F. W., George, G. A.:* Int. J. Biochem. 10, 275—277 (1979).
[69] *Hendrickson, H. S., Fullington, J. G.:* Biochemistry 4, 1599—1605 (1965).
[70] *Jackowski, M. M., Johnson, R. A., Exton, J. H.:* Biochim. Biophys. Acta 630, 497—510 (1980).
[71] *Jacobus, W. E., Tiozzo, R., Lugli, G., Lehninger, A. L., Carafoli, E.:* J. Biol. Chem. 250, 7863—7870 (1975).
[72] *Jeng, A. Y., Shamoo, A. E.:* J. Biol. Chem. 255, 6897—6903 (1980).
[73] *Jerzmanowski, A., Staron, K.:* J. Theoret. Biol. 82, 41—46 (1980).
[74] *Johnson, J. H., Pressman, B. C.:* Arch. Biochem. Biophys. 132, 139—145 (1969).
[75] *Juzu, H. A., Holdsworth, E. S.:* J. Membrane Biol. 52, 185—186 (1980).
[76] *Kagawa, K., Kurohata, M., Tomizawa, S.:* Biochem. Biophys. Res. Comm. 83, 1299—1305 (1978).
[77] *Kanagasuntheram, P., Lim, S. C.:* Biochem. J. 176, 23—29 (1978).
[78] *Karl, R. C., Zawalich, W. S., Ferrendelli, J. A., Matschinsky, F. M.:* J. Biol. Chem. 250, 4575—4579 (1975).
[79] *Klee, C. B., Crouch, T. H., Richman, P. G.:* Ann. Rev. Biochem. 49, 489—515 (1980).
[80] *Krämer, R.:* Biochim. Biophys. Acta 592, 615—620 (1980).
[81] *Kramer, G. L., Hardman, J. G.:* in Handbook of Physiology. Section 2: The Cardiovascular System, Vol. II. ed. Bohr, D. F., Somtigo, A. P., Sparks Jr., H. V., p. 179—199 Waverley Press, Inc. Baltimore, Md (1980).
[82] *Kun, E.:* Biochemistry 15, 2328—2336 (1976).
[83] *Lehninger, A. L., Vercesi, A., Bababunmi, E. A.:* Proc. Natl. Acad. Sci. 75, 1690—1694 (1978).
[84] *Ligeti, E., Horvath, L. I.:* Biochim. Biophys. Acta 600, 150—156 (1980).
[85] *Loud, A. V.:* J. Cell Biol. 37, 27—46 (1968).
[86] *Lötscher, H. R., Winterthaler, K. H., Carafoli, E., Richter, C.:* Eur. J. Biochem. 110, 211—216 (1980).
[87] *Lucas-Lenard, J., Lipmann, F.:* Proc. Nat. Acad. Sci. USA 57, 1050—1057 (1976).
[88] —, —: Ann. Rev. Biochem. 40, 409—448 (1971).
[89] *MacIntyre, I., Davidson, D.:* Biochem. J. 70, 456—462 (1958).

[90] *Maguire, M. E., Erdos, J. J.:* J. Biol. Chem. 255, 1030—1035 (1980).
[91] *Malmström, B. G., Rosenberg, A.:* Adv. Enzymol. 21, 131—167 (1959).
[92] *Manner, G., Kuleba, M.:* Connective Tiss. Res. 2, 167—176 (1974).
[93] *Martindale, L., Heaton, F. W.:* Biochem. J. 92, 119—126 (1964).
[94] *McCarthy, B. J.:* Biochim. Biophys. Acta, 55, 880—888 (1962).
[95] *McDonald, J. M., Bruns, D. E., Jarett, L.:* Proc. Natl. Acad. Sci. USA 73, 1542—1564 (1976).
[96] *Merker, H. J., Günther, T.:* Z. Klin. Chem. Klin. Biochem. 8, 71—76 (1970).
[97] *Miyamoto, H., Racker, E.:* J. Biol. Chem. 255, 2656—2658 (1980).
[98] *Mukherjee, C., Caron, M. G., Lefkowitz, R. J.:* Proc. Natl. Acad. Sci, 72, 1945—1949 (1975).
[99] *Murphy, E., Coll, K., Rich, T. L., Williamson, J. R.:* J. Biol. Chem. 255, 6600—6608 (1980).
[100] *Nelson, D. L., Kennedy, E. P.:* J. Biol. Chem. 246, 3042—3049 (1971).
[101] —, —: Proc. Nat. Acad. Sci, USA 69, 1091—1093 (1972).
[102] *Newton, C., Pangborn, W., Nir, S., Papahadjopoulos, D.:* Biochim. Biophys. Acta 506, 281—287 (1978).
[103] *Nicholls, D. G.:* Biochem. J. 176, 463—474 (1978).
[104] *Nicholls, D. G., Crompton, M.:* FEBS Letters 111, 261—268 (1980).
[105] *Nishimura, S., Harada, F., Hirabayashi, M.:* J. Mol. Biol. 40, 173—186 (1969).
[106] *Quigley, G. J., Teeter, M. M., Rich, A.:* Proc. Nat. Acad. Sci. USA 75, 64—68 (1978).
[107] *Page, E., Polimeni, P. I.:* J. Physiol. (London), 224, 121—139 (1972).
[108] *Papahadjopoulos, D., Portis, A.:* Ann. N. Y. Acad. Sci. 308, 50—66 (1978).
[109] *Park, M. H., Wong, B. B., Lusk, J. E.:* J. Bacteriol. 126, 1096—1103 (1976).
[110] *Philipson, K. D., Nishimoto, A. Y.:* J. Biol. Chem. 255, 6880—6882 (1980).
[111] *Pörschke, D.:* Nucleic Acids Res. 6, 883—898 (1979).
[112] *Porzig, H.:* J. Membr. Biol. 31, 317—349 (1977).
[113] *Piascik, M. T., Wisler, P. L., Johnson, C. L., Potter, J. D.:* J. Biol. Chem. 255, 4176—4181 (1980).
[114] *Ramirez, F., Marecek, J. F.:* Biochim. Biophys. Acta 589, 21—29 (1980).
[115] *Rasmussen, H., Goodman, D. B. P.:* Physiol. Rev. 57, 421—509 (1977).
[116] *Rayssiguier, Y., Larvor, P.; Durlach, J.:* Compt. rend. seances Soz. Biol. 168, 736 (1974).
[117] *Rayssiguier, Y., Larvor, P., Augusti, Y., Durlach, J.:* Ann. Biol. anim. Biochem. Biophys. 17, 147—152 (1977).
[118] *Ribero, J. M. C., Vianna, A. L.:* J. Biol. Chem. 253, 3153—3157 (1978).
[119] *Rogers, T. A., Mahan, P. E.:* Proc. Soc. Exp. Biol. Med. 100, 235—239 (1956).
[120] *Rogers, T. A., Haven, F. L., Mahan, P. E.:* J. Nat. Cancer. Inst. 25, 887—888 (1960).
[121] *Romero, P. J.:* Biochim. Biophys. Acta, 339, 116—125 (1974).
[122] *van Rossum, G. D. V.:* J. Gen. Physiol. 55, 18—32 (1970).
[123] *Rothman, J. E., Lenard, J.:* Science 195, 743—753 (1977).
[124] *Sanui, H., Rubin, H.:* J. Cell Physiol. 92, 23—32 (1977).
[125] *Schilling, K., Börnig, H., Cumme, G., Hoppe, H.:* Acta biol. med. germ. 39, 177—184 (1980).
[126] *Schilling, K., Preukczas, St., Börnig, H.:* Acta biol. med. germ. 38, 1101—1114 (1979).
[127] *Schimmel, R. J.:* J. Cyclic Nucleotide Res. 5, 327—338 (1979).
[128] *Schmalbeck, J., Willems, W. R., Dorn, F., Günther, T.:* J. Clin. Chem. Clin. Biochem. 10, 270—274 (1972).
[129] *Seimiya, T., Ohki, S.:* Biochim. Biophys. Acta, 298, 546—561 (1973).
[130] *Shlatz, L., Marinetti, G. V.:* Science, 176, 175—177 (1972).
[131] *Siliprandi, O., Toninello, A., Zoccarato, F., Siliprandi, N.:* Biochem. Biophys. Res. Comm. 78, 23—27 (1977).
[132] *Silver, S., Clark, D.:* J. Biol. Chem. 246, 569—576 (1971).
[133] *Simpson, I. A., Pfeuffer, T.:* Eur. J. Biochem. 111, 111—116 (1980).
[134] *Sordahl, L. A.:* Arch. Biochem. Biophys. 167, 104—115 (1974).
[135] *Späh, F., Fleckenstein, A.:* J. Mol. Cell Cardiol. 11, 1109—1127 (1979).
[136] *Stein, A., Crothers, D. M.:* Biochemistry 15, 157—160 (1976).
[137] *Subramanian, A. R., Ron, E. Z., Davis, B. D.:* Proc. Nat. Acad. Sci, USA 61, 761—767 (1968).
[138] *Szer, W., Ochoa, S.:* J. Mol. Biol. 8, 823—834 (1964).
[139] *Tada, M., Ohmori, F., Yamada, M., Abe, H.:* J. Biol. Chem. 254, 319—326 (1979).
[140] *Takahashi, K., Weiner, H.:* J. Biol. Chem. 255, 8206—8209 (1980).
[141] *Takeda, Y.:* Biochim. Biophys. Acta, 182, 258—261 (1969).
[142] *Veloso, D., Guynn, R. W., Oskarsson, M., Veech, R. L.:* J. Biol. Chem. 248, 4811—4819 (1973).
[143] *Vitale, J. J., Nakamura, M., Hegsted, D. M.:* J. Biol. Chem. 228, 573—576 (1957).
[144] *Wallach, D., Pastan, I.:* Biochem. Biophys. Res. Comm. 72, 859—865 (1976).
[145] *Weber, L. A., Hackey, E. D., Haroney, P. A., Baglioni, C.:* J. Biol. Chem. 252, 4007—4010 (1977).
[146] *Weller, D. B., Shechter, Y., Musgrave, D., Rougvie, M., Horowitz, J.:* Biochemistry 7, 3668—3675 (1968).
[147] *Whang, R., Welt, L. G.:* J. Clin. Invest. 42, 305—313 (1963).