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Review

Magnesium
An update on physiological, clinical and analytical aspects

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Abstract

There is an increased interest in the role of magnesium ions in clinical medicine, nutrition and physiology. The characteristics of the binding of magnesium and calcium ions to various components, macromolecules and biological membranes are described. Magnesium affects many cellular functions, including transport of potassium and calcium ions, and modulates signal transduction, energy metabolism and cell proliferation. The mechanism of cellular uptake and efflux of magnesium, its intracellular transport, intestinal absorption, renal excretion and the effect of hormones on these are reviewed. Magnesium deficiency is not uncommon among the general population: its intake has decreased over the years especially in the western world. The magnesium supplementation or intravenous infusion may be beneficial in various diseased states. Of special interest is the magnesium status in alcoholism, eclampsia, hypertension, atherosclerosis, cardiac diseases, diabetes, and asthma. The development of instrumentation for the assay of ionized magnesium is reviewed, as are the analytical procedures for total magnesium in blood and free magnesium in the cytosol. The improved procedures for the assay of different magnesium states are useful in understanding the role of magnesium in health and disease.

Abbreviations: Ca, calcium, calcium ion; Mg, magnesium, magnesium ion; P_i, inorganic phosphate; PTH, parathyroid hormone

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1. Introduction

Magnesium (Mg) is the main intracellular earth metal cation with a free concentration in the cytosol around 0.5 mmol/l [1–5]. Cytosolic free [Ca^{2+}] is only about 1/10 000 of the corresponding extracellular species, traditionally called ionized calcium. Calcium (Ca) therefore is an ideal agent for fast signal transduction and activation of a cell. It is evident that Mg, whose gradient over the plasma membrane is slight, and whose free extracellular concentration (ionized Mg) is about 0.7 mmol/l, at most can play the complementary role of a more long-term regulatory element [1,3,4]. Nevertheless, with the recent developments in analytical methods and instrumentation for measuring both ionized and cytosolic free Mg concentrations (see the section on analytical aspects) it has been possible to gain a better insight into the physiology of Mg. Here, we review also the clinical and analytical aspects of Mg research.

2. Chemistry and biochemistry of Mg

In order to understand the behavior of Mg, it is useful to recall some basic facts about it. In contrast Ca, Mg is a smaller ion that attracts water molecules more avidly. Thus in practice, the ion is quite large [4–6]. Its six coordination bonds also have more rigid coordination distances and directions than the more flexible Ca with its six to eight coordination bonds [4]. In contrast to Ca, Mg binds to neutral nitrogen groups such as amino-groups and imidazol in addition to oxygen especially in acidic groups, while calcium binds to oxygen in multidentate anions [4]. As a result, magnesium binding to protein and other molecules generally is weaker than that of calcium, and it is more difficult for it to reach and adapt to more deeply-situated binding sites in proteins [7], and to pass through narrow channels in biological membranes. This may also be the reason for the difficulty in finding probes that are highly Mg-specific.

Mg is a cofactor in hundreds of enzymatic reactions [3,8–11] and is especially important for those enzymes that use nucleotides as cofactors or substrates. This is because, as a rule, it is not the free nucleotide but its Mg complex that is the actual cofactor or substrate. This is true for phosphotransferases and -hydrolases such as ATPases which are of central importance in the biochemistry of the cell, particularly in energy metabolism. In addition, Mg is required for protein and

nucleic acid synthesis, the cell cycle, cytoskeletal and mitochondrial integrity and for the binding of substances to the plasma membrane [8,10]. Mg frequently modulates ion transport by pumps, carriers and channels [5,10–12] and thereby may modulate signal transduction and the cytosolic concentrations of Ca and potassium.

Positively charged Mg^{2+} is able to bind electrostatically to the negatively charged groups in membranes, proteins and nucleic acids. The binding to membrane phospholipid head-groups may change the local conformation and have a general electrical screening effect [13]. Accordingly, Mg may influence the binding of other cations like Ca and polyamines [9] which, depending upon their concentration, may have antagonistic or cooperative effects. Generally, Mg has a membrane-stabilizing and protecting effect, which may be due to the electrical effects [13] and to inhibition of phospholipase A_2 (EC 3.1.1.4) [9].

In many biochemical reactions, Mg can be partially, or in some cases fully, replaced by polyamines, spermine (four positive charges), spermidine (three charges) or the diamine, putrescine [14]. Thus, both Mg and polyamines bind efficiently to the negatively charged groups in membranes, nucleic acids and ribosomes [15,16]. The two types of cations are involved in the synthesis of DNA, RNA and proteins. However, an unresolved question is this: if Mg and polyamines can perform the same or similar functions, which type of cation is actually operative in the cell in a particular physiological condition? Furthermore, what does determine the cellular choice of the particular alternative actually used? Polyamines with their charges separated at fixed lengths may be the preferred molecular species whenever the structural complexity of the interacting species requires it, as in the stabilization of DNA. There is also experimental evidence for substitutive effects. Thus, Mg deficiency in the rat produces an increase in the spermidine content of brain cortex [17].

3. Physiology of Mg

It has long been known that Mg is important for normal neurological and muscular function, hypomagnesemia leads to hyperexcitability due mainly to cellular Ca transport and signalling [1–3,8]. The adult body contains approximately 21–28 g (about 1 mole) of Mg, muscle and soft tissues accounting for almost half of this and bone for slightly more than half [1]. Only about 1% of Mg is present in the blood plasma and red cells.

3.1. Control of cytosolic free Mg

Though the gradient of free Mg over the plasma membrane is modest, it may vary and thereby influence various cellular activities. The main factors affecting cytosolic free Mg are the concentration of nucleotides and operation of transport

systems in the plasma membrane and mitochondria. Especially important is ATP, which binds Mg with an association constant of around 4 [4], while the binding affinity is about two orders of magnitude less for ADP. The cytosolic free Mg thus would rise in cells in a poor energy state with less ATP [2], as in anoxia [18], and the same is true for free Mg in the mitochondrial matrix [19]. One special case is the erythrocyte where also 2,3-bisphosphogluconate and hemoglobin are significant Mg buffers. The model for the variation of total and free Mg with the oxygenation state of hemoglobin — the free Mg increases with deoxygenation — has recently been refined [20].

Mg influx into the cell occurs mainly by diffusion from the slightly higher free concentration in the extracellular space [1,2,5,8,10,11]. This is promoted by the membrane potential, negative on the cytosolic side. There are also data supporting channel-mediated influx in cardiovascular and epithelial cells [1,2], though not via Ca channels since their blockers had no effect [11]. One possibility is electroneutral symport with anions such as hydrogencarbonate [21].

Since efflux of Mg occurs against the electrochemical gradient, there must be an energy-coupled mechanism for the extrusion of Mg. This is achieved mainly by antiport against Na^+, at least in erythrocytes, hepatocytes, squid axons and ascites cells [5,8,10,11,22], i.e. on the expense of the Na^+ gradient. Most studies favor an electroneutral antiport mechanism of $2Na^+/Mg^{2+}$ [5], in which case the energy input would be solely from the Na^+ gradient. Also in smooth muscle cells the Na^+-dependence argues in favor of the antiport mechanism [23] while for heart cells other explanations for this dependence have been put forward [24]. However, there is an ATP-dependence which has been interpreted as indicating a contribution from a Mg pump mechanism; also protein phosphorylation or binding could be involved [5,10,11,24]. Mg may also influence the activity of protein kinases, thus the elevated Mg in erythrocytes seen with deoxygenation of hemoglobin is associated with tyrosin phosphorylation of band 3 [25].

Mitochondrial Mg transport also influences Mg_i. The gradient over the inner membrane is small but the energy state — phosphorylation potential and the membrane potential — influences the fluxes. There is a respiration-driven Mg influx or efflux depending on the external $[Mg^{2+}]$ and other experimental conditions [26,27]. The mechanism of this Mg transport is not clear, but a leak mechanism, influenced by the free Mg gradient and the membrane potential appears likely [6]. The matrix free Mg is also changing with the metabolic state: inorganic phosphate and ATP reduce the free Mg while a conversion of ATP to ADP increases it [19]. The influx is stimulated by inorganic phosphate and inhibited by K^+ and by quinine, but in contrast to Ca, not by La^{3+} or ruthenium red [28]. In liver and kidney, but not in the heart, there is also an antiport mechanism of $Mg^{2+}ATP^{2-}/P_i^{2-}$, that increases the total Mg but does not change matrix free Mg but may have a modulatory effect on the adenine nucleotide

content since the antiporter is activated by Ca [29]. Matrix free Mg regulates oxidative phosphorylation and affects the K^+/H^+ antiporter that controls the mitochondrial volume together with K^+ uniport [30]. Of special importance is the inhibition by Mg of the swelling and uncoupling of mitochondria that have taken up Ca over a certain threshold [31]. This mitochondrial dysfunction is due to the opening of a large pore in the inner membrane and is called the mitochondrial permeability transition [32]. The probability of pore opening is increased by Ca and decreased by Mg [33]. This is of special interest since this is one way in which Mg can protect cells from the harmful effects of irreversible opening of the pore that may occur in various pathological conditions [34–36]. Tumor cells frequently have a high content of Mg which may inhibit the permeability transition [37].

Efflux of Mg from mitochondria occurs by a separate mechanism [6,11] possibly by antiport against H^+ [38] or K^+ [27], for review see Ref. [39]. Substances that increase the matrix free Mg, like lowering of matrix ATP by exchange against ADP, also stimulate efflux [6]. The antiporter $Mg^{2+}ATP^{2-}/P_i^{2-}$ may also contribute to efflux of Mg since it is reversible and P_i stimulates efflux [6,11,28].

The endo- and sarcoplasmic reticulum may also be involved to some extent in Mg handling with Mg serving as a counterion for Ca in the calcium pump, at least under some conditions [11].

3.2. Transcellular transport of Mg

Transcellular Mg transport takes place mainly in intestinal absorption and renal excretion [10].

3.2.1. Intestinal absorption of Mg

Mg is absorbed mainly in the ileum and in the colon [1,2,10,40]. The absorption is primarily by a passive paracellular mechanism dependent upon solvent drag [1,2,10], but models of passive leak and active extrusion to the serosal side are also possible [40], and are likely in view of the mechanisms for cellular transport of Mg outlined above. The passive leak is supported by the finding that there is a largely linear relationship between Mg concentration in the lumen and its absorption though there are indications of a saturation [40] which however may be due to binding of Mg and not to its transport. More recent studies with brush-border vesicles using Mag-fura-2 confirm the involvement of the $2Na^+/Mg^{2+}$ antiporter in the basolateral membrane in the intestinal Mg absorption [41]. However, the Na^+-dependence may well be due to the activity of the Na^+,K^+-2Cl^-symporter that would provide Cl^- to a $Mg^{2+}-2Cl^-$ symporter that is the actual mechanism of Mg extrusion [42].

3.2.2. Renal Mg excretion

Approximately 75% of the total plasma Mg is filtered through the glomerular membrane. In contrast to Na^+ and Ca, only 15% of the filtered Mg is reabsorbed in the proximal tubules, most (50–60%) in the thick ascending loop of Henle [1,2]. Under normal conditions only 3–5% of the filtered Mg is excreted in the urine [2].

As in the mucosa, both the paracellular pathway and epithelial transport are important in the tubular reabsorption of Mg which varies extensively with the filtered load. Several drugs, particularly diuretics, thiazides, cisplatin, gentamycin and cyclosporin cause Mg loss into the urine by inhibiting the Mg reabsorption in the kidneys [1,2]. The thiazide-sensitive Na^+–Cl^- symporter in the distal convoluted tubule is implicated as being involved since in the rat its amount correlates closely with dietary Mg intake, plasma ionized Mg and urinary excretion of Mg [43]. In analogy with the mucosa, its function could be to provide Cl^- to a Mg^{2+}–$2Cl^-$ symporter.

The mechanism of the paracellular transport of Mg has remained elusive but now rapid progress is to be expected. A study of a rare genetic disease with Mg wasting — renal hypomagnesemia with hypercalciuria and nephrocalcinosis — has identified a mutated gene, *paracellin-1*, coding for a protein located in the tight junctions of the thick ascending limb of Henle [44].

3.3. Hormonal modulation of Mg

Despite early proposals for the existence of a specific hormonal control of Mg homeostasis [2,45,46], our understanding of the endocrine factors that control circulating or urinary Mg is incomplete. Among many extensive and excellent reviews dealing with Mg homeostasis, one describes Mg as body's 'orphan' ion, because of an apparent lack of a specific endocrine control similar to that exists for Ca, sodium and potassium [47]. The cellular availability of Mg is closely regulated by the kidney, the gastro-intestinal tract and bone [48], the kidney being the main organ responsible for the regulation of Mg. A number of hormones including parathyroid hormone (PTH) and calcitonin, vitamin D, insulin, glucagon, antidiuretic hormone, aldosterone and sex steroids have been reported to influence Mg balance [47–49], notwithstanding the possibility that these may not be the primary regulators of Mg homeostasis. Some of the actions of selected hormones that affect cellular Mg are briefly outlined here. The reader is referred to the excellent reviews published on this subject [2,46–49].

3.3.1. Hormonal effects on intracellular Mg

In many tissues, hormones affect the cellular Mg content, mainly by modulation of Mg efflux that is energy-coupled (see Section 3.1). This process

evidently can be influenced by hormones via their intracellular messengers, frequently the cytosolic free Ca or cAMP. The findings are diverse due to the different parameters studied in different tissues. Both α_1-adrenergic agonists such as phenylephrine and β-adrenergic agonists like norepinephrine or isoproterenol stimulated Mg efflux in cardiac and liver cells [11]. Angiotensin II lowered the cytosolic free Mg in vascular smooth muscle cells of rats with genetic hypertension [50]. However, vasopressin caused accumulation of Mg in cardiac myocytes [26]. Also in ascites cells cAMP was stimulatory [22]. In perfused liver it was found that treatment with glucagon and phenylephrine caused an accumulation of both Mg and Ca in the mitochondria [51].

3.3.2. Calciotropic hormones and Mg

The key hormones that regulate the amounts of Ca, phosphate and Mg are PTH, vitamin D and calcitonin [46]. Their actions are similar for Ca and Mg. The calciotropic hormones exert their influence on Mg in the kidney, affecting Mg reabsorption in the cortical part of the thick ascending limb of the loop of Henle and in the distal convoluted tubules by different cellular mechanisms [2,47–49].

PTH stimulates Mg reabsorption both in the loop of Henle and in the distal tubule [2,52,53]. The PTH modulation of Mg is mediated by activation of adenylate cyclase and production of cAMP [52]. Calcium may modulate the PTH action as in primary hyperparathyroidism when Mg reabsorption is impaired due to a large renal Ca load resulting in hypermagnesuria [54]. There are reports that PTH also releases Mg from bone [55] and increases its absorption in the small intestine [54,55]. Also, Mg levels may influence PTH secretion through a feedback system, thus chronic hypermagnesemia may suppress PTH secretion and cause disturbances in Ca homeostasis [47,56–58].

Vitamin D has been shown to enhance the intestinal absorption of Mg through separate active transport mechanisms [55,59]. However, this phenomenon may not play an important role in the overall Mg homeostasis because of an increased urinary excretion [60]. Recently it has been shown that in hypoparathyroid patients, long-term treatment with vitamin D results in reduced renal Mg excretion while the Ca balance was positive [61].

Little is known about the effects of calcitonin on Mg. It has been reported to stimulate renal Mg reabsorption in the rat [62]. It activates adenylate cyclase in different parts of the nephron than PTH, i.e. in the medullary and the cortical portion of the thick ascending limb and in the bright portion of the distal tubule [52].

3.3.3. Steroid hormones and Mg

During the menstrual cycle, a cyclic variation in the Ca/Mg ratios was reported [63]. There was a significant decrease in the level of ionized Mg at the

time of ovulation; total Mg however decreased only in the luteal phase. This indicates a role for estrogens and progesterone in Mg homeostasis. This may be due to the inhibitory effect of estrogen on PTH-induced bone resorption, resulting in reduced plasma total Ca and stimulation of PTH secretion [64]. In women ionized Mg decreased also with increasing testosterone levels [63].

Aldosterone has little effect on renal Mg secretion, but chronic administration results in renal Mg wasting due possibly to volume expansion [1,46,49] and/or potassium depletion [46]. Incubation of immortalized mouse distal convoluted tubule cells with aldosterone alone had no effect on Mg uptake but it potentiated glucagon- and arginine vasopressin-stimulated Mg uptake rate, possibly though intracellular signalling pathways involving cAMP [65]. There is some evidence that Mg may modulate aldosterone production by adrenal cells in vitro [66].

3.3.4. Effects of insulin, glucagon and vasopressin on Mg

3.3.4.1. Insulin

Several studies have shown that among patients with diabetes mellitus the frequency of hypomagnesemia is higher than expected, and that it is correlated with the degree of severity of hyperglycemia (see Section 4.1.5). In healthy humans however, glucose-loading did not affect total or ionized plasma Mg although there were expected changes in circulating glucose, insulin, potassium and ionized Ca [67]. Results obtained in vitro with human platelets indicate that insulin may enhance cellular Mg uptake in a dose-, time- and receptor-dependent manner [68]. In vivo, the hormone acting on the loop of Henle can decrease the excretion of Mg [49]. At variance with this finding Corica et al. [69] have reported a reduction in plasma Mg concentration and an elevation of erythrocyte and platelet Mg levels in healthy humans after a 75 g oral glucose tolerance test.

3.3.4.2. Glucagon

In almost all respects, the actions of glucagon are exactly opposite to those of insulin. In healthy humans there was no change in circulating ionized or total Mg following glucagon injection in healthy humans [70]. In the rat, glucagon has been shown to increase Mg reabsorption both in the loop of Henle [49,71,72] and in the superficial distal tubule [73]. The effects of glucagon and arginine vasopressin were additive in their action [74]. The actions of glucagon are mediated by adenylyl cyclase (EC 4.6.6.1) and cAMP [49]. Glucagon receptors are present in the rat distal tubule [75] and it has been shown that glucagon stimulates adenylate cyclase in isolated distal tubule cells indicating that glucagon may act, in part, through cAMP [76]. Glucagon and arginine vasopressin stimulated Mg uptake in immortalized distal convoluted tubule cells in a concentration-dependent manner; it was inhibited by a Ca channel blocker

and was abolished by protein kinase A inhibition, indicating a role for cAMP [77].

3.4. Mg intake

Whole seeds, unmilled grains, green leafy vegetables, legumes and nuts are the richest dietary sources of Mg. The Mg, which is present in the unprocessed foods, is almost completely lost during the processing of food items [78]. Phytate, fibre, alcohol, or an excess of phosphate and Ca^{2+} attenuate the absorption of Mg [7], presumably by lowering its concentration in the lumen. Fish, meat, milk and fruits are generally poor sources of Mg [78]. Mg in drinking water has been suggested to account for only about 10% of the daily Mg intake [79], but can markedly vary, since considerable differences in water Mg content are found in different geographical areas [79,80]. The recommended dietary allowance (RDA) for Mg is 350 mg per day for a male adult and 280 mg per day for a female [78]. The Mg requirement is increased during pregnancy and lactation (355 mg/day) [78]. Human milk containing about 30–40 mg Mg/l is believed to provide adequate Mg for the growing infant [78].

There is evidence that the daily Mg intake has declined substantially since the beginning of this century, when it was estimated to be 475–500 mg [81]. Recent dietary surveys have shown that the average Mg intake in western countries is often below the RDA [82].

4. Clinical aspects of Mg

4.1. Mg deficiency as a risk factor

The important role of Mg in modulating transport functions and receptors, signal transduction, enzyme activities, energy metabolism, nucleic acid and protein synthesis as well as protecting biological membranes makes Mg deficiency a potential health hazard.

The development of Mg deficiency is usually linked either to disturbances in the intestinal Mg absorption and/or to an increased renal Mg excretion. In gastrointestinal disorders like intestinal malabsorption, steatorrhea and chronic pancreatic insufficiency, non-absorbable magnesium-fatty acid soaps may be formed [83]. Factors increasing renal Mg excretion are discussed earlier (see Sections 3.2.2 and 3.3).

Anorexia, nausea, vomiting, lethargy and weakness are typical early symptoms of Mg deficiency. If severe Mg deficiency develops, paresthesia, muscular cramps, irritability, decreased attention span and mental confusion often occur.

The physical signs of Mg deficiency are largely due to the associated hypocalcemia and hypokalemia [83].

There is an accumulating body of evidence to suggest that dietary Mg deficiency plays an important role in the pathogenesis of ischemic heart disease, congestive heart failure, sudden cardiac death, cardiac arrhythmias, vascular complications of diabetes mellitus, pre-eclampsia/eclampsia and hypertension [84,85]. Several studies have also been able to show a salutary effect of Mg supplementation in the treatment of the above-mentioned diseases.

4.1.1. Mg and pre-eclampsia/eclampsia

Magnesium sulfate was used for the first time in the prevention of eclamptic seizures as early as 1906 [86]. At present, it is widely used as a routine therapy to prevent eclamptic seizures in pregnant women with hypertension. The Collaborative Eclampsia Trial provided compelling evidence in favor of the use of $MgSO_4$, rather than diazepam or phenytoin, in the treatment of eclampsia [87,88]. Treatment of mothers with $MgSO_4$ before delivery might also reduce the risk of cerebral palsy and mental retardation in early preterm infants [89–92].

4.1.2. Mg and stroke

A recently published prospective study among 43 738 US men (Health Professional Follow-Up Study) demonstrated an inverse association between dietary Mg intake and the risk of total stroke. The inverse association was stronger in hypertensive than normotensive men and was not materially altered by adjustments for blood pressure levels [93].

Mg has been shown to be neuroprotective in several experimental models of ischemic and excitotoxic brain injury [94–98]. The possible mechanisms of neuroprotection include noncompetitive blockade of the NMDA receptor [99], enhanced regional cerebral blood flow to ischemic areas [100], inhibition of the Ca entry into the cells through leak, voltage-operated and receptor-operated channels, and favorable recovery of cellular energy metabolism after restoration of perfusion [85,101]. That this may be due to the inhibition by Mg of the mitochondrial permeability transition is important [31–33,36]. A large multicenter trial assessing the role of intravenous $MgSO_4$ treatment after acute stroke is now in progress [102], and this study should define whether or not $MgSO_4$ should be included in the treatment of acute stroke.

4.1.3. Mg and ischemic heart disease

Experimental and epidemiological studies have linked hypertension, hypertensive heart disease and ischemic heart disease with the use of soft water containing little amount of Mg [78,79,85]. Results from autopsy studies have shown lower myocardial and skeletal muscle total Mg in decedents who died

from ischemic heart disease as compared to those who died from accidents [78]. Mg deficiency has been shown to deteriorate hypokalaemia, cause cardiac arrhythmias and to expose to digitalis-induced side-effects [79,85,103]. Furthermore, Mg deficiency induces severe vascular damage in the heart and kidney, accelerates the development of atherosclerosis, causes vasoconstriction of the coronary arteries, increases blood pressure and induces thrombocyte aggregation [103].

Infusion of Mg, when given at pharmacological concentrations, produces vasodilatation of systemic vasculature and coronary arteries, platelet inhibition and antiarrhythmic effects [101]. Infusion of Mg also effectively protects myocardium against ischemia-reperfusion injury in experimental animals [104].

The second Leicester Intravenous Magnesium Intervention Trial (LIMIT-2) was the first randomized, double-blind, placebo-controlled study demonstrating that intravenous Mg therapy has a protective effect during the treatment of acute myocardial infarction [105,106]. Mg infusion, which was administered prior to or in parallel with thrombolytic agents, improved both early and long-term outcome, and it also significantly reduced the incidence of left ventricular failure in patients with acute myocardial infarction [105,106].

In a more recent prospective study where the effects of early oral captopril, oral mononitrate, and intravenous $MgSO_4$ were assessed among 58 050 patients with suspected acute myocardial infarction (ISIS-4), no beneficial effects were observed with Mg therapy [107]. It should be pointed out that in the ISIS-4 study Mg was administered after iatrogenic or spontaneous reperfusion, and therefore the difference in timing of the Mg therapy can explain, at least in part, the unexpected poor therapeutic results [108,109]. Further clinical studies are clearly needed before Mg can be considered as a routine therapy in patients with suspected myocardial infarction.

4.1.4. Mg and hypertension

Epidemiological studies have shown an inverse relationship between dietary Mg intake and the level of blood pressure [110–112]. However, the evidence is inconsistent and many of the clinical studies are methodologically imperfect and based on small study population [110].

Magnesium supplementation has been shown to decrease blood pressure in several [113–116], but not in all clinical studies [117–120]. Resnick et al. [121] were the first to describe a strong inverse relationship between erythrocyte cytosolic free Mg and diastolic blood pressure. With the use of ion-selective probes and NMR spectroscopic techniques, several research groups have now been able to confirm that cytosolic free Mg is usually lower in vascular smooth muscle and circulating blood cells of hypertensive compared to normotensive subjects [85,122].

4.1.5. Mg and diabetes mellitus

A strong association between Mg, diabetes and hypertension has been described. Cytosolic free Mg is frequently low in diabetic patients [85,122,123]. Magnesium deficiency aggravates insulin resistance and predisposes diabetic subjects to cardiovascular diseases. On the other hand, it has been shown that oral Mg supplementation improves the control of diabetes [124–127]. Hence, these data support the view that Mg supplementation might be of particular benefit in hypertensive diabetic subjects. However, in a recent extensive prospective study — the latest Atherosclerosis Risk in Communities (ARIC) Study [128]; it was found that low dietary Mg intake did not confer increased risk for type 2 diabetes in a middle age population. There was though a clear inverse correlation between serum total Mg levels and the incidence of diabetes in the white, but not in the black population. In an editorial comment to the ARIC study [129], doubt is expressed on the causal relationship between low serum Mg and the risk for diabetes; the low Mg could be due to increased Mg loss in the urine.

4.1.6. Mg and atherosclerosis

In experimental animals, dietary Mg deficiency exacerbates atherosclerosis and vascular damage [85,103]. In cholesterol-fed animals oral Mg supplementation lowers serum cholesterol and triglycerides and attenuates the development of atherosclerotic lesions [85,103]. Magnesium deficiency is often associated with a number of dyslipidemias. The available data support the notion that dietary Mg intake plays an important modulatory role in controlling lipid metabolism in the arterial wall [85].

4.1.7. Mg and asthma bronchiale

Dietary Mg intake has been shown to be independently related to lung function, airway reactivity, and respiratory symptoms in the general population [130]. The salutary effect of Mg are apparently brought about mainly by competition with Ca entry through voltage and receptor-operated Ca channels as well as by the inhibition of intracellular Ca release from sarcoplasmic reticulum. However, inhibition of cholinergic transmission, stimulation of the synthesis of nitric oxide and prostacyclin, and stabilization of mast cells and T lymphocytes could also be responsible, at least in part, for the beneficial effects of Mg in asthma. Even though $MgSO_4$ has been shown to cause bronchodilatation and improve lung functions [131,132], the use of Mg in the treatment of asthmatic patients remains to be determined.

4.1.8. Mg in other pathological conditions

Possible involvement of Mg in migraines [133], osteoporosis [134], alcoholism [135] and immune system disorders [136] has also been suggested. See also ‘Relevance of the assay of ionized Mg’ (Section 5.2.3.2).

4.2. Mg toxicity

The therapeutic window of Mg is wide, and in the absence of renal failure, severe side-effects are extremely rare. Oral Mg supplementation can cause mild side-effects like diarrhea and abdominal cramps. Early signs of Mg toxicity during intravenous Mg treatment include vomiting, nausea, feeling of warmth, flushing, hypotension, bradycardia and other cardiac arrhythmias, somnolence, double vision, slurred speech and weakness [83,135]. These side-effects usually occur at total plasma Mg of 3.5–5 mmol/l. Hyporeflexia (loss of patella reflex), muscular paralysis, respiratory arrest and cardiac arrest develop only at extremely high plasma Mg concentrations (5–15 mmol/l) [83,133]. Magnesium toxicity is exaggerated in the presence of hypocalcemia, hyperkalemia, and uremia. Calcium gluconate serves as an effective antidote for Mg toxicity [83,137].

5. Analysis of Mg

In plasma Mg, like Ca, can be found in three fractions; in an ultrafiltrable fraction consisting of ionized Mg (70–80%), complex-bound Mg (1–2%) and in a protein-bound non-ultrafiltrable fraction (20–30%) [138]. The reference range for total Mg concentration in adult blood plasma is 0.65–1.05 mmol/l [139], for ionized Mg 0.55–0.75 mmol/l [140], and for total Mg in erythrocytes 1.65–2.65 mmol/l [139].

In current clinical laboratories, Mg is measured predominantly as total substance concentration. Determination of this parameter is routinely, though not as frequently as for total Ca, requested for blood serum or plasma, or as daily excretion in urine. Mg deficiency is often diagnosed using hair as the sample. On some occasions determination of total Mg in erythrocytes is requested.

Despite the fact that ionized fractions of sodium, potassium and Ca are now most frequently requested in routine clinical analysis, until recently the ionized Mg was not covered by any direct method, although this fraction is the one that possesses the biological activity. A relevant method for measurement of ionized Mg was developed in the beginning of the nineties and is now gaining the status of a routine method in clinical analysis.

5.1. Assay of total Mg

Determination of total Mg in a variety of human samples is available by a variety of techniques. Photometry using a number of chromogenic reagents such as xylidyl blue, calmagite, methyltymol, magon and titan yellow are most frequently used [139,141]. Another frequently used technique is atomic spectroscopy in two modes: flame emission (FEAS) or absorption after electrother-

mal atomization (AAS). The latter is especially useful in the analysis of hair. Occasionally, inductively-coupled plasma (ICP) optical emission is used, especially when a multicomponent, serial analysis of biological liquids is requested. In all of these techniques, it is possible to obtain data with a relative standard deviation of 1–3%.

Several authors have reviewed the importance and clinical relevancy of the assay of total Mg in physiology and medicine [142,143].

5.2. Assay of ionized Mg

Owing to the chemical properties of Mg, determination of ionized Mg has been difficult and demanding, and existing ion-selective electrodes have suffered from a lack of selectivity and relatively long response times.

5.2.1. Principle of the assay

Ionized Mg is measured potentiometrically using a Mg-selective electrode, which together with a reference electrode forms an electrochemical system. In all instruments the measuring system consists of a series of electrodes in a flow-through block where ionized Mg, sodium, potassium, pH, chloride, ionized Ca and other electrolytes can be simultaneously measured from a single sample.

The Mg-selective electrode is of the liquid membrane type with a neutral carrier molecule dispersed in a plastic matrix together with additives and plasticisers. Corrections for Ca — and to a smaller extent, sodium — interference are performed automatically by the software of an instrument according to accepted chemometric strategies.

5.2.2. Development and evaluation of analyzers

The first successful implementation of a Mg-selective electrode in a flow-through instrument (Microlyte 6, KONE Instruments, Finland) was achieved in 1990 [144,145]. Then followed an instrument for the fully automated measurement of ionized Mg in human blood serum and plasma at the end of 1992 [138]. This instrument made it possible to measure in 2 min, in addition to ionized Mg, also ionized Ca, sodium, potassium, chloride and pH in a 150-μl sample. The linearity range for ionized Mg was 0.2–3.0 mmol/l, the inaccuracy was $<3\%$ and imprecision $<2\%$. The lifetime of the Mg sensors was more than 1000 samples.

In the beginning of 1994, the Finnish Microlyte Mg Analyzer [146] was no longer the only instrument available, since similar electrolyte analyzers were produced by other companies, i.e. the 988-4 Magnesium Analyzer from AVL (Austria) [147] and the Stat Profile 8 from NOVA (USA) [148]. Since then, there have been improvements, especially regarding the selectivity, throughput,

precision and lifetime of the electrodes, but the methodology has remained essentially the same.

The calibration is performed using aqueous standard solutions containing salts in concentrations corresponding to physiological ranges. Typically, the electrodes are calibrated using a three-point calibration, in the ionized Mg measurement additional verification of the Ca-selectivity coefficient $(K_{MgCa})_i$ is performed. The signal-to-concentration slope of Mg-electrodes in the calibrants without interfering ions is reported to be nearly Nernstian, i.e. close to 27 mV per decade [149]. The first independent evaluation of the instrument by KONE was reported by van Ingen et al. [150]. Soon after, the instruments produced by AVL and NOVA [151] as well as those by NOVA and KONE [152] were compared, and recently all three instruments were evaluated simultaneously by two independent laboratories [153].

5.2.3. Assay of ionized Mg in patient samples

Adoption of the sensor for clinical tests demanded a careful analysis of the chemical status of ionized Mg in serum, its reference values and sampling procedures. This task was not an easy one because of the apparent lack of standardized methods. Basically, recommendations concerning measurement of ionized Ca were adopted [154,155]. The following recommendations concerning sampling and sample treatment could be formulated [156]:

(1) Ionized Mg can be measured in whole blood, serum or plasma from venous blood samples.

(2) Sampling should preferably take place without a tourniquet and with the patient sitting, not supine. Any muscular action like ‘pumping’ should be avoided.

(3) As in the case of ionized Ca, changes in pH, due to loss of CO_2, will influence the complexation equilibrium of Mg in serum. Therefore an anaerobic handling with the use of vacutainers is recommended. Samples should be collected into non-coated vessels, glass tubes or vacutainers etc., that do not contain silicone. If plasma is needed, vessels should be heparinized without the use of silicone. Silicone is a major interfering substance that causes deterioration of the functioning of the Mg-selective electrode [146,147].

(4) The influence of heparin should be attributed to the binding of Mg to heparin [157]. As anticoagulant, sodium, lithium or potassium-titrated heparin can be used in concentrations up to 40 UI/ml (error $< 1.5\%$) [140] or even higher concentrations if Ca/Mg titrated heparin is applied [157]. The lack of heparin effect for several commercial containers containing heparin was reported as well [158].

(5) If serum samples are employed, these should be allowed to coagulate for 30 min before centrifugation. If plasma samples are used, a careful mixing is necessary immediately after collection in order to insure proper anticoagulation.

(6) Centrifugation should be performed using a relative centrifugal force of $2000 \times g$ for 10 min. After centrifugation the supernatant fraction should be separated from cells as soon as possible. Serum samples can be stored for up to 1 month at $+4$°C.

Pooled serum obtained from healthy and ill adults have given values of ionized Mg in the range 0.45–0.75 mmol/l. The most frequently seen values for healthy adults are 0.52–0.59 mmol/l and the ratio of ionized to total Mg was reported to be in the range 60–80% [156,159]. In another study the ratio of ultrafiltered to total Mg was found to be about 5% higher than the ratio of ionized to total Mg [160]. Consistently higher values obtained with ultrafiltration are obviously due to the presence of low-molecular mass, ultrafiltrable Mg complexes.

The influence of CO_2-induced pH change was found to be of similar nature to that found by Siggaard-Andersen for ionized Ca and as in the case for ionized Ca may be characterised with dimensionless coefficient X. The increase of pH decreases the value of ionized Mg with a significantly smaller coefficient; $X = -0.09$ for Mg in comparison to $X = -0.21$ for Ca [140,156,157].

5.2.3.1. Future work

The assay of ionized Mg could be further improved with respect to the sensitivity (low Mg) [161], selectivity (interference by Ca) [138] and non-specific interferences (silicone [146,147], detergents [162], thiocyanate in smokers [163]).

Work in pursuit of improving the method is underway in numerous laboratories. Improvements in sensor selectivity [164] and in resistance to detergents used during washing [162] were recently reported.

5.2.3.2. Relevance of the assay of ionized mg

Despite reported difficulties, assaying of ionized Mg is gaining popularity. This is obviously due to the numerous reports on the relevance of ionized Mg in different clinical situations and the superiority of this parameter over total Mg. This concerns bowel syndrome [159], cardiac surgery [165], ischemic or hemorrhagic stroke [166,167], atherogenic lipid fractions [168], renal dysfunction [169], continuous ambulatory peritoneal dialysis (CPAD) [170,171], alcoholism [172,173], liver disease [169,174], eclampsia [175], neonatal hypomagnesemia [176], and hemodialysis [177].

5.3. Assay of cytosolic free Mg

A number of techniques are available for the assay of cytosolic free Mg_i. These include Mg-selective electrodes, metallochromic indicators, null point for plasma membrane permeabilization, ^{31}P nuclear magnetic resonance spec-

trometry (NMR), and fluorescent probes. The use and drawbacks of these techniques have been reviewed by Romani and Scarpa [11]. The main difficulty is the interference from cytosolic free Ca, Na^+, and pH, especially for metallochromic indicators. For some of these techniques very specialized instrumentation is needed which makes them unavailable for most clinical laboratories. Still, the NMR technique, if available, is quite specific. It relies on the differences in the spectra of ATP and its Mg complex. It also compares favorably with the zero-point titration method for erythrocytes [178].

5.3.1. Fluorescent probes

The development of fluorescent indicators similar to those for cytosolic free Ca have made it possible to measure cytosolic free Mg both in cell suspensions using a two-excitation wavelength fluorometer and in individual cells with a microscope connected to a fluorometer. The probe widely used is mag-fura-2 ([18,19,21,41], and see below), for which the ratio of fluorescence with two excitation wavelengths is measured. Since the cytosolic free Ca is several orders of magnitude smaller than the cytosolic free Mg, the interference is usually negligible. One advantage of mag-fura-2 is that its affinity for Ca is much higher than for Mg, and that the Mg K_D, 1.9 mmol/l, is not appreciably changed between pH 5.5 and 7.4 [179].

Platelets are often used in the measurement of cytosolic free Mg with mag-fura-2 [180,181]. Another fluorescent Mg probe is mag-indo-1 that has been used for assay of cytosolic free Mg in mononuclear blood cells and erythrocytes [182]. Mag-indo-1 has also been used in flow cytometry of lymphocytes [182].

The probe penetrates the plasma membrane as an ester that is hydrolysed in the cytosol. However, this should be checked because the ester may have partially been able to penetrate also into cellular organelles such as mitochondria, thereby responding also to matrix Mg. Some of the free indicator may also be released from the cell and report ionized Mg. One source of error in patient samples can be the presence of fluorescent drugs. Interfering fluorescent material may also originate from dialysis membranes during plasmapheresis (Saris, unpublished observation). This can be checked by running fluorescence spectra before loading cells with the probe. Owing to the competition by polyamines for binding to ATP and other Mg-binding sites, changes in the concentration of polyamines during the cell cycle may seriously interfere with the measurement when using NMR or fluorescent probes [183].

5.4. Assessment of the Mg status

The new techniques for measuring ionized and cytosolic free Mg give useful information on the state of Mg in the body. Such assays may be good substitutes

for or complements to the determination of total Mg, see above. The small fraction of Mg present in the blood (1%) may be a limitation. Renal excretion of Mg seldom gives useful additional information due to the great influence of the amount of Mg in the diet and the many factors that affect the urinary Mg excretion. A reference range of 2.2–5.0 mmol Mg/day for females and 3.3–6.3 for males has been reported [184]. In severe Mg deficiency the daily excretion frequently is below 0.5 mmol/day. Increased Mg retention after loading tests indicates Mg deficiency [184]. Intravenous loading tests are reliable but more laborious [185].

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