Tab. 2: Mg content of diet, noise level and aging rates derived from the collagen increase in rat hearts.

Mg content of diet	Noise level	Aging rate
mmol/kg	dB	—
80	—	1
80	92	1
12	—	1.5
12	83	3
2	—	5
2	83	9

Mg deficiency increases the release and effect of catecholamines during noise exposure.

Under constant treatment conditions, the loss of Mg and the increase of the catecholamine level will accelerate in the final stage and lead to a shortened life span. This is in agreement with results of *Heroux* et al. (1977) who found that none of their rats under marginal Mg intake plus cold stress did reach the planned termination date of their experiment, whereas the life span of rats under a single treatment was not shortened.

From these results one can conclude that even mild chronic Mg deficiency combined with stress can accelerate the aging process, so that a marked shortage of the life span will result.

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Magnesium effect on the permeability of the amniotic membrane as a whole and of the amniotic epithelial cells*)

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Zusammenfassung

Mg hat einen unterschiedlichen Effekt auf die Amnion-Membran, je nachdem, auf welcher Seite es angreift. An der menschlichen Amnion-Oberfläche ist Mg unter in-vitro-Bedingungen auf der foetalen Seite ein Inhibitor monovalenter Kationen und verursacht einen Wasserverlust in die interzellulären Kapillaren; auf der mütterlichen Seite erhöht Mg den Transfer monovalenter Kationen, lockert das Membranretikulum auf und erhöht die Motilität monovalenter Kationen, innerhalb der Membran, indem es auf die Phosphoryl-Brücken einwirkt.

Bei ansteigenden extrazellulären Mg-Konzentrationen wird die Membran der Amnionepithelzelle depolarisiert, und der Membranwiderstand nimmt ab. Mg wirkt auf die Quantität der aktiven Na-Kanäle und auf die interzelluläre elektrische Kopplung.

Summary

Mg has a different effect according to the side of the amniotic membrane it is acting on. On the fetal side, Mg is a competitive inhibitor of monovalent cations at the level of the human amnion surface sites *in vitro*, bringing about water loss in the intercellular channels, whereas on the maternal side, Mg increases the transfer of the monovalent cations, loosening the membrane reticulum, acting on the bridges connecting the phosphoryl groups and increasing the motility of the monovalent cations inside the membrane.

*) Results presented at the 3rd International Symposium on Magnesium, Baden-Baden, 22.—28. 8. 1981.

When the Mg external concentration increases, the membrane of the epithelial cells gets depolarized and the resistance (input resistance) of the membrane decreases. The quantity of active sodium channels depends on the Mg concentration; Mg acts on the intercellular coupling.

Résumé

Mg a un effet différentiel sur l'amnios selon le côté d'adjonction: du côté foetal, il agit comme un inhibiteur compétitif des cations monovalents au niveau des sites de surface de l'amnios *in vitro*, en provoquant une perte en eau dans les canaux intercellulaires, tandis que du côté maternel, il active le passage des cations monovalents en provoquant un relâchement du réticulum de la membrane et augmente la mobilité au sein de celle-ci par action sur les ponts entre les groupes phosphoryl.

Quand la concentration externe en magnésium augmente, la membrane des cellules épithéliales est dépolarisée et la résistance de la membrane diminue. Le magnésium agit sur la quantité de canaux sodiques actifs et sur le couplage électrique intercellulaire.

* * *

Introduction

The human amnion is a fixed neutral sites membrane (negative sites in certain physiological and experimental conditions). The monovalent cations permeate through the amnion (gK > gNa) and the mother to fetus conductance is 3 to 4 times that of the fetus to mother conductance [7]. Furthermore, the paracellular pathway through epithelial cells is more important than transcellular pathway and there is an electrical intercellular coupling [2].

We have studied the effect of Mg^{++} (at different concentrations in extracellular maternal liquids and in amniotic fluid) on the monovalent cations transfer across paracellular and transcellular pathways. That is why Mg^{++} is added on the maternal side or on the fetal side and the effects on the electrical parameters of the amniotic membrane as a whole as well as of the epithelial cell membrane, are measured.

Material and Methods

— Strips of human amnion, isolated from the placental (ZP), reflected (ZR) and umbilical (ZUp, ZUf) zones of the amniotic sac, were obtained after delivery or cesarean sections term pregnancies. The amniotic membranes were immersed in *Hanks'* solution (pH = 7.4 and at 37 ± 1°C). A circular area of amnion ($2cm^2$) was sampled and put in between two Ussing chambers for the whole study or between two Lucite chambers for epithelial cells study. Temperature was maintained constant by passing electrical current through a heating plate around the chambers.

— Amniotic conductance was measured by observing the transepithelial potential difference (p.d.) when a direct current (usually 100 mAp) was passed across the whole tissue. The p.d. was recorded with two agar salt bridges placed 1.3 —1.5 mm from each side of the tissue, while electrical current passed across the tissue by means of Ag/AgCl electrodes and agar salt bridges and was measured on a Schlumberger electrometer. The "edge" conductance associated with the damaged ring of tissue clamped between the chambers is negligible compared to the conductance of the rest of the tissue, since the same value of conductance per unit area, was obtained whether the chambers had an exposed area of 1 cm^2 or of 2 cm^2.

— The membrane potential (Vm) was measured with intracellular glass microelectrodes filled with 3M KCl inserted into the epithelium under visual control using a binocular microscope and a micromanipulator. Vm, between the microelectrode tip and the Ag/AgCl indifferent electrode in the bath, was measured with a high input impedance amplifier (Pico-metric Amplifier 181, Instr. Lab. Inc.) and an oscilloscope (Tektronix 502A). The input resistance (Rin) was measured by injecting electrical current, square-wave pulse through a double barrelled microelectrode and analysing the recorded transient voltage.

— A second microelectrode was impaled into another cell of the same strand and located at a distance varying between 100—500 microns, away from the site of current injection. Measurements of space constant (λ) were made by recording the amplitude of the electrotonic potentials generated by a constant pulse of current at different distances from the polarizing electrode (100—500 μ) and plotting them against the distance in a semilogarithmic scale.

Separate values of r_m (membrane resistance per unit length) and r_i (intracellular longitudinal resistance per unit length) were calculated using the following equations: $r_m = 2\ Rin\ \lambda$ and $r_i = 2\ Rin/\lambda$. The electrical coupling factor was calculated by V_2/V_1 (voltage in cell 1 and in cell 2 after the passage of current).

— Solutions: *Hanks'* solution used contained (mM/l): NaCl 137, KCl 5.4, $CaCl_2$ 1, $MgSO_4$ 0.5, $MgCl_2$ 0.5, Na_2HPO_4 0.22, KH_2PO_4 0.35, $NaHCO_3$ 3.4, glucose 5.5.

— Mg^{++} was added to the saline solutions to give a final concentration of 10 mM/l for the study of amniotic membrane as a whole and 12 mM/l for epithelial cell membrane study.

Results

1. Study of the whole membrane

The amniotic membrane is bathed with *Hanks'* solution (1 mM Mg^{++}) or by monovalent solution (100 mM NaCl or KCl, pH 7.4). Progressively, magnesium concentration is increased, on one side of the membrane and the transmembrane potential, the dilution potential, the bi-ionic (K-Na) potential, the conductance and the flux across the amnion are measured at different concentrations of Mg^{++} on the amniotic cavity side (ACS) or on the maternal side (MS).

a) Transmembrane potential (Table 1)

The effect of Mg^{++} is different when the membrane is bathed with *Hanks'* or NaCl solutions. The transmembrane potential is more great in NaCl solution than in Hanks' solution (5 to 6 times much important). Also, the interactions between divalent cations and membrane, and between divalent and monovalent cations are more important when the membrane is bathed with NaCl solution than with *Hanks'* solution. When Mg^{++} is added on the ACS, the potential becomes negative.

At the 30—50 mM/l magnesium concentration, the potential reaches a saturation plateau and there is a high and significative correlation between magnesium concentration and transmembrane potential ($r = 0.85$, $p < 0.01$).

b) Bi-ionic potential (Bip)

Magnesium concentration is 2 or 10 mM/l on ACS or MS and the potential sign shows that K^+ is more permeant than Na^+. At 2 mM/l of Mg^{++}, Bip is unvariable, but it increases of 8 % (Mg^{++} on ACS) and of 33 % (Mg^{++} on MS) at 10 mM/l.

c) Dilution potential

The experimental conditions are: X^+Cl (100 mM)/membrane/X^+Cl (10 mM) ($X^+ = Na^+$ or K^+); the magnesium concentration on MS or on ACS is 2 or 10 mM/l.

Whatever sample area, we have two effects: — at 2 mM/l, the dilution potentials decrease of 11 % when Mg^{++} is added on ACS and of 28 % on MS. The anionic transfer number (t^-) increases and the cationic transfer number (t^+) decreases; — at 10 mM/l, the dilution potentials increase to 8 % when Mg^{++} is added on ACS and to 25 % on MS, and t^+ increases.

d) Conductance and flux through the amnion

The unidirectional flux from the internal to the external medium is given by *Goldman*'s equation:

$$J_i^{out} = \frac{P_j z F \Delta\Psi/RT}{1 - \exp(-zF \Delta\Psi/RT)} C_i$$

with P_j = permeability of j ion (here, P_j = conductance of j ion: g_j)
C_i = internal ionic concentration
$\Delta\Psi$ = $\Psi_i - \Psi_e$ = transmembrane potential (Ψ_i and Ψ_e are the electrical potentials in respectively internal and external solutions)
z, F, R, T = regular constants

The results are expressed in Table 1 and show that the mother to fetus flux is more important than the fetus to mother flux.

— When the Mg^{++} concentration is less than 2 mM/l on ACS or on MS, the monovalent cations conductance decreases to 30 %. When the Mg^{++} concentration is more than 2 mM/l on MS, the conductance increases but does not vary or decreases slightly when Mg^{++} is added on ACS.

— The curves $1/g$ = function of $[Mg^{++}]_e$ or $1/[Mg^{++}]_e$ (*Dixon*'s enzymatic kinetics) show that Mg^{++} is a competitive inhibitor of monovalent cations on the ACS and an activator of monovalent cations transfer on the MS (Figure 1).

2. Study of epithelial cells membrane (Table 2)

a) — The membrane was hyperpolarized by removing Ca^{++} from the medium. The membrane polarity was not held by 1 mM Mg^{++}. Removal of Mg^{++} ions from the bathing medium significantly increased Vm, but the hyperpolarization is less important than when Ca^{++} is removed.

— An excess of Mg^{++} ions (1 to 12 mM/l) depolarizes the membrane. Regression analysis of the data obtained between 1 and 12 mM/l gives a linear plot (regression coefficient $r = 0.75$ for ZP, 0.68 for ZR, 0.63 for ZUp and 0.57 for ZUf (p 0.01) with a slope of 1.22 mV/mM for ZP,

Tab. 1: Action of Mg^{++} on transepithelial potential and flux through amniotic membrane (number of experiments n = 25).

	Δ V (mV)/10 mM Mg^{++}				J_i^{out} (μM)	
	Mg^{++} on amniotic cavity side		Mg^{++} on maternal side		Mg^{++} on amniotic cavity side	Mg^{++} on maternal side
	Hanks	NaCl	Hanks	NaCl		
ZP	−1.75 ± 0.20	8.4 ± 0.2	3.2 ± 0.1	15.9 ± 0.3	90	1786
ZR	−1.60 ± 0.06	8.1 ± 0.6	2.9 ± 0.1	14.5 ± 0.7	96	1550
ZUp	−0.30 ± 0.05	9.3 ± 0.5	3.6 ± 0.05	16.8 ± 0.4	113	2186
ZUf	−0.12 ± 0.08	9.9 ± 0.4	3.9 ± 0.07	18.3 ± 0.5	128	2488

Tab. 2: Action of Mg^{++} on electrical parameters of amniotic epithelial cell membrane (number of experiments n = 22).

	Vm (mV)		Rin (10^3 ohms)		λ (mm)		10^2 r_m (ohms.cm)		10 r_i (ohms.cm^{-1})		V_2/V_1	
	control	+12 mM/l Mg^{++}	control	+12 mM/l Mg^{++}	control	+12 mM/l Mg^{++}	control	+12 mM/l Mg^{++}	control	+12 mM/l Mg^{++}	control	+12 mM/l Mg^{++}
ZP	−18.3 ± 0.7	−6.2 ± 0.5	213 ± 7	142 ± 4	2.3 ± 0.2	3.3 ± 0.3	980 ± 10	937 ± 6	185 ± 4	86 ± 3	0.78	0.84
ZR	−15.3 ± 0.4	−5.6 ± 0.4	172 ± 6	132 ± 5	2.4 ± 0.2	2.8 ± 0.2	825 ± 8	739 ± 6	143 ± 5	94 ± 3	0.80	0.88
ZUp	−12 ± 0.4	−5.3 ± 0.3	160 ± 4	123 ± 4	1.8 ± 0.3	2.2 ± 0.2	576 ± 7	541 ± 7	178 ± 4	112 ± 4	0.70	0.75
ZUf	−8.3 ± 0.3	−5.2 ± 0.2	138 ± 4	106 ± 3	1.9 ± 0.3	2.3 ± 0.2	524 ± 7	488 ± 6	145 ± 5	120 ± 4	0.70	0.75

0.84 mV/mM for ZR, 0.65 mV/mM for ZUp and 0.35 mV/mM for ZUf).

b) Adding an excess of Mg^{++} ions induced a significantly decrease in input resistance. At 12 mM/l of Mg^{++}, Rin is divided by 1.5 for ZP, 1.3 for ZR, ZUp and ZUf. (Figure 2).

c) At 12 mM/l of Mg^{++}, λ and intercellular coupling (V_2/V_1) increase; r_m and r_i decrease.

Discussion and conclusion

a) — Mg^{++} has an identical effect for the 4 zones of the amniotic membrane.

— The potentials are diffusion potential on MS and interfacial potentials on ACS (observation of time constants).

— Mg^{++} increases the intercationic selectivity and the cationic transfer number at large concentrations. At low concentrations, the effect of Mg^{++} is due to a cationic conductance decrease [8].

— Mg^{++} has a differential effect according to the side of the amniotic membrane: on the fetal side, Mg^{++} is a competitive inhibitor of the monovalent cations at the level of the human amnion

surface sites, bringing out water loss in the intercellular channels, whereas on the maternal side, Mg^{++} increases the transfer of monovalent cations loosening the membrane reticulum, acting on the bridges connecting the phosphoryl groups and increasing the motility of the monovalent cations inside the membrane.

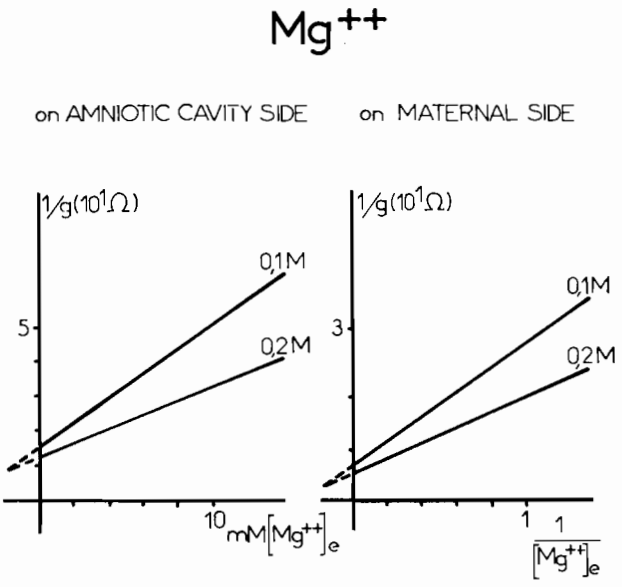

Fig. 1: 1/g in function of $Mg^{++}{}_e$ or $1/Mg^{++}{}_e$ (Dixon's enzymatic kinetics).

also contribute to the resting potential since removal of calcium or magnesium induces cell hyperpolarization and conductance decreases.

An excess of Mg^{++} induces cell depolarization and a reduction of input resistance. It is difficult to determine how magnesium contributes to the membrane potential. However, one may think of

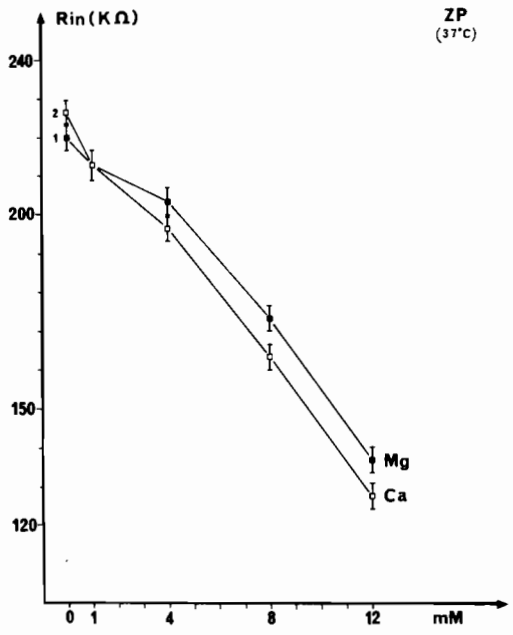

Fig. 2: Action of Mg^{++} on input resistance Rin (comparative action of Ca^{++} is given).

— The differential effect of Mg^{++} suggests that the sites are different on the maternal and the fetal sides.

b) An interpretation of the ionic effects on membrane potential and input resistance of amniotic cells is difficult because there is no information about the intracellular mobilities of different ionic species or about the membrane permeability to different ions. Indeed, we know that Na^+ diffuses across the human amnion [3] [4] and that divalents cations, in the bathing medium, increase membrane permeability [6]. In the amnion, ionic gradients to Na^+ and K^+ establish the membrane potential as in excitable or non-excitable cells [1]. Calcium and magnesium three possibilities: 1 – regulating the viability of sodium or potassium channels, thus allowing a more or less efficient flux of monovalent cations; 2 – Mg^{++} may contributes directly to the membrane potential if it is actively pumped in or out across the cell membrane [5]; 3 – an action on the electrical intercellular coupling since Mg^{++} reduces the intracellular longitudinal resistance and increases the factor coupling (V_2/V_1).

c) Also, Mg^{++} has an effect on the transcellular and paracellular pathways and on the intercellular coupling.

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Behavioral and biological effects of oral magnesium, vitamin B6 and combined magnesium — vitamin B6 administration in autistic children*) **)

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Zusammenfassung

Es wird über Verhalten und biochemische Parameter von 52 autistischen Kindern berichtet, die unter Doppelblindbedingungen im Überkreuzversuch folgendermaßen behandelt wurden: A: Vit. B_6 + Mg; B: Magnesium; C: Vit. B_6. Die therapeutischen Effekte wurden einerseits mittels Rating-scales and andererseits anhand der Ausscheidung von Homovanillinmandelsäure (HVA) im Urin beurteilt.

Nach Gabe von Vitamin B_6 + Mg wurde eine Besserung des Verhaltens und eine Normalisierung der HVA-Ausscheidung beobachtet. Nach alleiniger Gabe von Vit. B_6 oder Mg wurden diese Wirkungen nicht gesehen.

Summary

This is a report of behavioral and biological effects of 3 therapeutic crossed sequential double-blind trials on 52 autistic children: Trial A (vitamin B6 + magnesium); Trial B (magnesium); Trial C (vitamin B6). Therapeutic effects were controled using behavior rating scales on one hand and urinary excretion of homovanillic acid (H.V.A.) on the other hand.

With vitamin B6 + Mg, a diminution of autistic symptoms is observed as well as a normalisation of urinary H.V.A. levels. These effects are not observed when vitamin B6 or Mg are administered alone.

Résumé

Les effets cliniques et biologiques du magnésium et de la vitamine B6 prescrits séparément ou associés sont étudiés. 52 enfants autistiques ont participé à 3 essais croisés doubleaveugle: Essai A (vitamine B6 + magnésium); essai B (magnésium); essai C (vitamine B6). L'appréciation des effets thérapeutiques est réalisée d'une part à l'aide d'échelles de comportement et d'autre part par le dosage de l'acide homovanilique urinaire (H.V.A.).

Lorsque Mg + B6 sont associés, on observe une amélioration clinique et une tendance à la normalisation des taux de l'H.V.A. urinaire. Ces effets ne sont par observés quand Mg et B6 sont prescrits séparément.

* * *

According to several studies [3, 17, 19], vitamin B6 has been found effective in the treatment of autistic children. The observed clinical improvement is associated with a decreased level of uri-

*) Results presented at the 3rd International Symposium on Magnesium, Baden-Baden, 22.—22. 8. 1981.

**) This study was supported by CNRS ERA (N-697) "Biological Methods in Neuropsychiatry", INSERM, ATP 78—97 and CL 79, Fondation pour la Recherche Médicale.