ELSEVIER

Rapid Communication

Modulating role of glucose on magnesium transport in rat erythrocytes

Gisèle Franck, Joanna Wdzieczak-Bakala, and Jean-Georges Henrotte

Institut de Chimie des Substances Naturelles, CNRS, Gif-sur-Yvette, France

Magnesium efflux from rat erythrocytes has been shown to be inhibited by a plasma fraction containing glucose. Therefore, we investigated the effect of D-glucose on erythrocyte magnesium transport. We show the inhibitory activity of this hexose on sodium (Na^+)-independent erythrocyte magnesium ($Mg^{2+}E$) efflux. Inhibitory effects of D-mannose, 2-deoxy-D-glucose, and D-fructose on $Mg^{2+}E$ efflux also were demonstrated. Moreover, the suppression of the inhibitory activity of glucose on $Mg^{2+}E$ efflux was shown to be associated with the inhibition of glucose transport by cytochalasin B and phloretin. Together these data suggest a possible implication of the glucose carrier GLUT-1 in the regulation of Mg^{2+} transport. (J. Nutr. Biochem. 10:433–437, 1999)

Keywords: magnesium; glucose; monosaccharides; GLUT-1; rat erythrocyte; membrane transport

Introduction

The physiologic importance of magnesium (Mg^{2+}), the second most abundant intracellular cation, is well recognized. Mg^{2+} plays a fundamental role in the structure, metabolism, and bioenergetics of the cell.[1–3] It has been shown to participate in over 300 enzymatic reactions in humans, particularly in those involving energy utilization.[4,5] Its implication in glycolysis, cell respiration, and transmembrane transport of other cations such as sodium, potassium, and calcium has been reported.[1,6] Magnesium also is required for protein and nucleic acid synthesis[7,8] and for a number of mitochondrial processes.[9] In view of the implication of magnesium in many fundamental functions of the cell, it is crucial that a constant intracellular level of this cation is maintained. Indeed, it has been reported that changes in cytosolic Mg^{2+} concentration lead to significant modifications of cell functions.[10] However, little is known about the regulation of the intracellular Mg^{2+} level in mammalian cells. Among the systems involved in the regulation of cellular Mg^{2+} homeostasis, the best known is the sodium (Na^+)-dependent Mg^{2+} transport. It accounts for approximately 80% of cellular Mg^{2+} efflux when the cells are loaded with Mg^{2+} and catalyzes outward Mg^{2+} movements in the presence of external Na^+ and internal adenosine triphosphate (ATP).[11,12] The existence of this kind of phenomenon has been reported in chicken,[11] human,[12] and murine[13] erythrocytes. The existence of another system of Mg^{2+} transport that is Na^+-independent also has been demonstrated.[14] It accounts for approximately 80% of cellular Mg^{2+} efflux when the cells are not loaded with Mg^{2+}. In this case the electroneutrality inside cell is maintained by chloride (Cl^-) efflux.

Our previous studies showed the ability of mammalian blood plasma to inhibit in vitro the Mg^{2+} efflux from rat erythrocytes.[15,16] This inhibition was attributed to thermostable low molecular weight compounds.[17] Recently, one of the isolated plasma fractions containing glucose revealed a significant inhibitory activity. Therefore, the effect of chemically pure D-glucose on in vitro rat erythrocyte Mg^{2+} ($Mg^{2+}E$) efflux was investigated. We report here the inhibitory effect of glucose on Na^+-independent $Mg^{2+}E$ efflux. We also suggest the possible implication of the glucose transporter GLUT-1 in the regulation of Mg^{2+} flux.

Address correspondence to Dr. Joanna Bakala, ICSN, CNRS, 91198 Gif-sur-Yvette Cedex, France.
This research was supported in part by a grant from the Ministère de l'Environnement (MENV no. 94081).
Received September 9, 1998; accepted April 14, 1999.

655 Avenue of the Americas, New York, NY 10010
0955-2863/99/$–see front matter
PII S0955-2863(99)00025-X

Material and methods

Animals

Male Sprague-Dawley rats, aged 3 to 4 months (300–400 g) were obtained from Janvier Laboratory (Le Genest St Isle, France). The animals were kept in our animal house for at least 10 days before experiments. They received tap water and regular food pellets (UAR, Villemoisson s/Orge, France) that were rich in Mg^{2+} (75 mmol/kg) ad libitum.

Materials

Rats were anesthetized with ether, and 15 mL of blood from the abdominal aorta were collected on heparin. Samples were immediately centrifuged at 4°C for 30 minutes at 2,150 g. Plasma and buffy coat were removed and the remaining pellet of erythrocytes was harvested. Mg^{2+}- and Ca^{2+}-free Dulbecco's phosphate buffered saline (PBS) containing 138 mM NaCl and 5 mM KCl was supplied by Gibco (Cergy Pontoise, France). α-D-Glucose, α-D-mannose, α-D-galactose, 1-O-methyl-α-D-glucose, 2-deoxy-α-D-glucose, α-D-glucose-1-phosphate, α-D-glucose-6-phosphate, α-D-glucose-1,6-phosphate, α-D-fructose, α-D-xylose, α-D-ribose, cytochalasin B, cytochalasin E, and phloretin were purchased from Sigma (Saint Quentin, Yvelines, France). Cytochalasin B, cytochalasin E, and phloretin solutions in ethanol were prepared prior to use. Strontium chloride hexahydrate extra pure, sodium chloride, and potassium chloride were obtained from Merck (Nogent-sur-Marne, France).

Experimental procedure

Two hundred fifty microliters of fresh packed erythrocytes were incubated at 37°C for 5 hours either in 750 μL of Mg^{2+}- and Ca^{2+}-free PBS (control) or in the same medium supplemented with various amounts of D-glucose (0.5–20 mM final concentrations) or of different monosaccharides (10 mM final concentration). In all cases the incubation media were adjusted at 295 mOsm prior to use. In another set of experiments the erythrocytes were incubated in the presence of either 10 μM cytochalasin B, 10 μM cytochalasin E, 100 μM phloretin, or the mixture of 10 μM cytochalasin B and 100 μM phloretin. In addition, incubations were carried out in the simultaneous presence of different hexoses (10 mM) and the above mentioned compounds. To evaluate the part of Na^+-independent and Na^+-dependent Mg^{2+}E efflux affected by glucose, the erythrocytes were incubated in isotonic phosphate buffer containing either both NaCl and KCl or KCl alone. The simultaneous presence of Na^+ and K^+ allowed the measurement of total Mg^{2+}E efflux, whereas the suppression of Na^+ from the incubation medium permitted determination of the Na^+-independent Mg^{2+} efflux. The Na^+-dependent Mg^{2+}E efflux was calculated from the difference between the values of the total and the Na^+-independent Mg^{2+} efflux. The effect of glucose on the Mg^{2+} efflux from Mg^{2+}-loaded erythrocytes also was evaluated. Briefly, fresh erythrocytes were washed twice with cold 150 mM NaCl, and incubated at 4°C for 17 hours with either 55 mM or 75 mM $MgCl_2$ adjusted with PBS up to 295 mOsm. Mg^{2+}-loaded erythrocytes were then washed four times with cold 150 mM NaCl (or 150 mM KCL). At the end of each incubation the samples were centrifuged twice (10 min, 1,100 g, 4°C); 30 μL of packed erythrocyte pellet were hemolyzed in 5 mL of 8.17 g/L $SrCl_2$, 6 H_2O solution. All hemolysates were prepared in triplicate. Mg^{2+} concentration in the obtained samples was determined by flame atomic absorption spectrophotometry following the recommendations of Stendig-Linberg et al.[18] The coefficient of variation was equal to 1.5%, emphasizing the high reproducibility of measurements.[19] To evaluate the rate of Mg^{2+} efflux, Mg^{2+}E contents were measured at time 0 and after 5 hours incubation. Correction of the eventual modulations of Mg^{2+}E level linked with variations in red cell volume during the incubation were performed using the values of hemoglobin concentration as described previously.[16]

The inhibition of Mg^{2+}E in the presence of monosaccharides in the incubation medium was calculated according to the formula:

$$\text{inhibition (\%)} = [(\text{efflux}_{\text{control}} - \text{efflux}_{\text{sugar}})/\text{efflux}_{\text{control}}] \times 100$$

in which $\text{efflux}_{\text{control}}$ and $\text{efflux}_{\text{sugar}}$ are the Mg^{2+} efflux determined in PBS without and with addition of the various monosaccharides tested, respectively. Because the level of erythrocyte Mg^{2+} and the values of Mg^{2+}E efflux showed important variations from one rat to another,[9] the calculations were performed systematically with the data obtained the same day with erythrocytes sampled from the same rat. The statistical significance of differences between means were estimated by one-tailed Student's *t*-test, computed on the differences between paired values. All results are expressed as means ± SEM.

Results and discussion

Inhibition of Mg^{2+}E efflux by glucose: Kinetics and mechanisms

Erythrocytes freshly sampled from six different rats were incubated separately in Mg^{2+}- and Ca^{2+}-free PBS containing Na^+ and K^+. In glucose-free medium (control not shown), total Mg^{2+}E efflux was equal to 154 ± 11 μM/(l cells × h). This efflux decreased with increasing concentration of glucose in the incubation medium. As shown in *Figure 1*, the inhibition of Mg^{2+}E efflux reached a maximal value (30 ± 6.5%) in the presence of 5 mM glucose. Further increase in glucose concentration did not alter this value. Furthermore, the curve reflecting the inhibitory effect of glucose on Mg^{2+}E efflux exhibits a Michaelian-like profile, which suggests the existence of an efflux facilitated by an accessory compound.

To further study this phenomenon, Na^+-dependent and Na^+-independent Mg^{2+} efflux were determined using three different pools of fresh erythrocytes. As shown in *Figure 2*, the addition of 10 mM glucose to the incubation media induced simultaneously a significant ($P < 0.05$) increase in Na^+-dependent and a decrease in Na^+-independent Mg^{2+} efflux. The inhibitory effect of glucose on the Na^+-independent efflux was further confirmed by another set of experiments performed on fresh and Mg^{2+}-loaded (55 and 75 mM) erythocytes. As shown in *Figure 3*, glucose induced 35% and 12% reduction, respectively, of Mg^{2+}

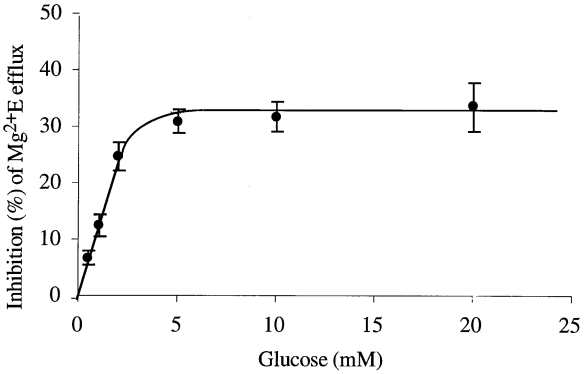

Figure 1 In vitro inhibition of erythrocyte magnesium (Mg^{2+}E) efflux from fresh erythrocytes incubated in Mg^{2+}- and calcium (Ca^{2+})-free phosphate buffered saline containing various concentrations of glucose (0.5–20 mM final concentrations). Glucose-free solution was used as control (inhibition=0). Results are means ± SEM of six different experiments.

efflux in non-loaded and mildly Mg^{2+}-loaded erythrocytes. In contrast, a 16% increase in Mg^{2+} efflux in heavily loaded cells was observed in the presence of glucose, strongly suggesting no inhibitory effect of this sugar on Na^+-dependent Mg^{2+} efflux. In fact, Mg^{2+} efflux has been recognized to be largely Na^+-dependent when the erythrocytes were loaded with the high concentration of Mg^{2+}.[11,12,20] These data are in accordance with the absence of inhibitory effect of glucose reported previously by a number of authors. In fact, it has to be precise that the former investigations were mostly carried out on Mg^{2+}-loaded erythrocytes.[11,12,20] In such conditions, the major mechanism involved in Mg^{2+}E efflux is a Na^+/Mg^{2+} antiport and its activity depends on the intracellular free Mg^{2+} concentration, the presence of ATP, and the external Na^+ concentration. After overnight loading, the erythrocyte energetic reserves are low and can be restored by the addition to the medium of glucose and/or ATP, which are able to increase the activity of the Na^+/Mg^{2+} exchanger. Taking into account these data, our findings show that, in Mg^{2+}loaded cells, glucose behaves as an activator of Mg^{2+}E efflux. This phenomenon overshadows the inhibitory effect of glucose on the Na^+-independent Mg^{2+} efflux, which can be observed only in fresh unloaded erythrocytes where the Na^+-independent mechanisms prevail.

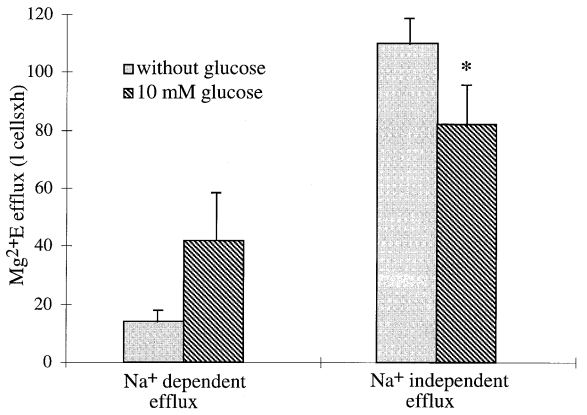

Figure 2 In vitro effect of glucose on sodium (Na^+)-independent and Na^+-dependent erythrocyte magnesium (Mg^{2+}E) efflux. Results are means ± SEM of three different experiments. *$P < 0.05$

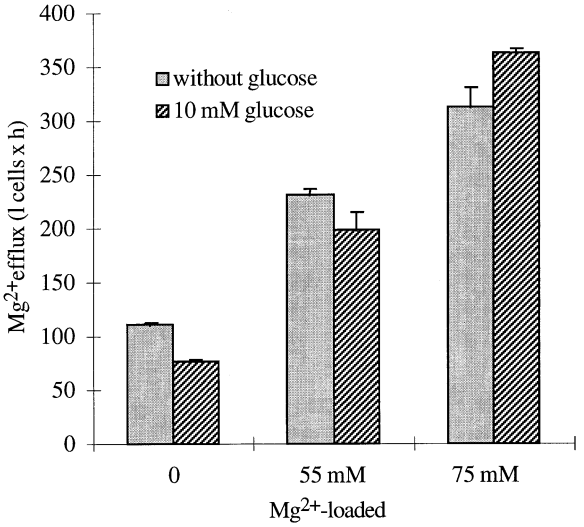

Figure 3 In vitro effect of glucose on total magnesium (Mg^{2+}) efflux from fresh and Mg^{2+}-loaded erythrocytes. Results are means ± SD of two different experiments performed on the same pool of erythrocytes obtained from two rats.

Inhibition of Mg^{2+}E efflux by monosaccharides and derivatives: Structure-activity relationship

The inhibitory activity of glucose on Mg^{2+} efflux was compared with that of other sugars with similar chemical structures. In *Table 1*, total Mg^{2+}E efflux measured in the absence (control) or in the presence of one of these compounds (10 mM) are indicated, as are their structural characteristics with reference to glucose. In *Figure 4*, the percentage of inhibition of Mg^{2+} efflux determined in the presence of these molecules is compared with that of glucose. As shown, none of the pentoses tested showed an inhibitory effect on Mg^{2+}E efflux. Furthermore, the inhibitory activity of hexoses disappeared when the hydroxyl groups were substituted at C-1, C-3, or C-6. Likewise, the equatorial position of the hydroxyl group is needed at C-4 because D-galactose, which is characterized by an axial hydroxyl group at C-4, did not display any significant inhibitory activity. In contrast, the presence or the position of the hydroxyl group at C-2 is irrelevant because 2-deoxy-D-glucose and D-mannose both inhibit of Mg^{2+}E efflux (*Table 1* and *Figure 4*), although D-mannose has an activity significantly ($P < 0.005$) lower than that of glucose (*Figure 4*). Moreover, the inhibitory activity of fructose was observed and its rate was shown to be lower ($P < 0.01$) when compared with that of glucose. The structural characteristics required for the monosaccharides to inhibit Mg^{2+}E efflux present some analogies with those considered to be critical for the transport of hexoses through the erythrocyte membrane.[21,22] Therefore, we decided to investigate the possible relationships between hexose transport and Mg^{2+} efflux.

Table 1 Effect of monosaccharides on magnesium efflux from fresh erythrocytes ($mg^{2+}E$)

	Monosaccharides	Structural characteristics	$Mg^{2+}E$ efflux	*P*-value
Control	—	—	118 ± 15	
Aldohexoses	D-glucose	—	75 ± 11	≤0.001
	D-galactose	C-4 epimer	111 ± 3	NS
	D-mannose	C-2 epimer	83 ± 12	≤0.005
Glucose derivatives	1-O-methyl α-D-glucose	OH substitution on C-1	114 ± 16	NS
	2-deoxy-D-glucose	No OH on C-2	92 ± 11	≤0.05
	3-O-methyl-D-glucose	OH substitution on C-3	116 ± 15	NS
	α-D-glucose-1-phosphate	OH substitution on C-1	121 ± 12	NS
	α-D-glucose-1,6-phosphate	OH substitution on C-3 and C-6	115 ± 14	NS
	α-D-glucose-6-phosphate	OH substitution on C-6	128 ± 15	NS
Ketohexoses	D-fructose	C-2 ketone	90 ± 10	≤0.01
Aldopentoses	D-xylose	—	116 ± 15	NS
	D-ribose	—	112 ± 14	NS

Results are means ± SEM of $Mg^{2+}E$ efflux in μmol/(L cells × h) from six different experiments. Incubation medium: Phosphate buffered saline (control) or PBS supplemented with 10 mM monosaccharide. Structural characteristics with reference to glucose (see text). *P*-value: level of significance of paired differences between experimental and control values (one-tailed Student's *t*-test).
NS–not significant.

Effects of GLUT-1 inhibitors on $Mg^{2+}E$ efflux

In the erythrocyte, glucose is transported by a carrier-mediated process that is not energy dependent and is not influenced by sodium or insulin.[23,24] GLUT-1, which is the transmembrane protein involved in this process, is potently and reversibly inhibited by a number of compounds that bind to the protein carriers much more tightly than does glucose. One of the most potent of these inhibitors is cytochalasin B, which preferentially blocks the uptake of glucose.[25] It binds to the transport system in the inner gated channel.[25,26] Cytochalasin E, which binds mainly to actin and spectrin and has no affinity for glucose carrier, was used as the negative control.[27,28] Glucose transport in the erythrocyte is also strongly inhibited by phloretin, which binds to the glucose carrier in the outer gated channel.[26] These data prompted us to examine the effect of cytochalasins E and B and phloretin on the inhibition of $Mg^{2+}E$ efflux by glucose. It must be mentioned that the addition of ethanol to the incubation medium at the same concentration as that present in cytocalasin and phloretin preparations had no effect on Mg^{2+} efflux from the treated erythrocytes (data not shown). As presented in *Table 2*, the presence of glucose in control media induced a diminution of Mg^{2+} efflux from 139 to 89 μmol/(l cells × h) (i.e., an inhibition of 36%). This latter value is very close to those given in *Figure 1* and *Table 1*, demonstrating the reproducibility of our results. The presence of cytochalasin E had no effect on the extent of this inhibitory action of glucose. In contrast, glucose tested in the presence of either cytocalasin B or phloretin, which are both glucose carrier inhibitors, was shown to loose 93% and 69%, respectively, of its inhibitory activity on Mg^{2+} efflux from the rat erythrocytes. The inhibitory effect of glucose

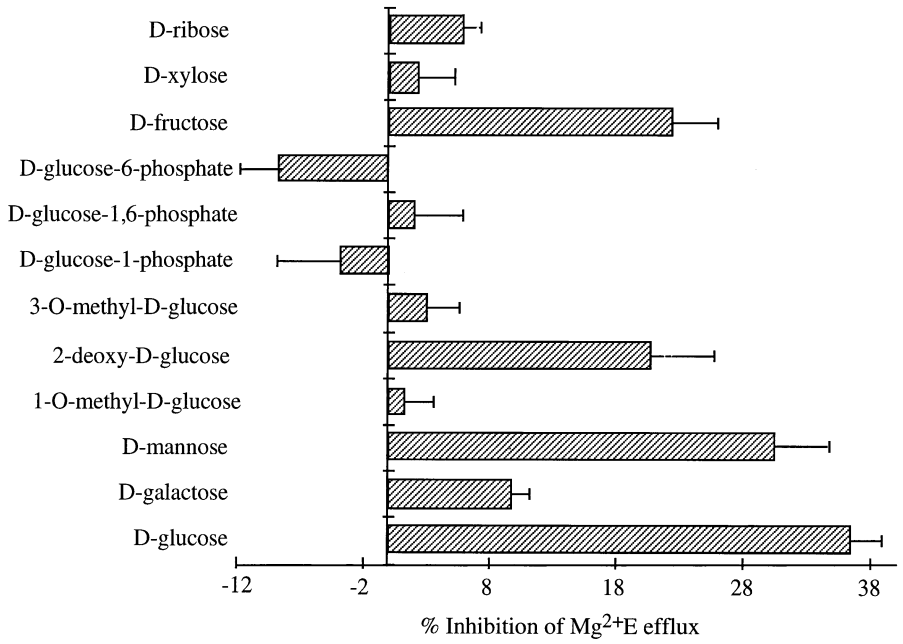

Figure 4 In vitro inhibition of erythocyte magnesium ($Mg^{2+}E$) efflux by various monosaccharides. Results are means ± SEM of six experiments.

Table 2 Effect of cytochalasins B and E and/or phloretin on magnesium efflux from fresh erythrocyte (Mg^{2+}E) incubated in the presence or absence of glucose

	Without D-glucose*	With D-glucose (10 mM)†	*P*-value‡
Control (PBS)	139 ± 5[a,b]	89 ± 2[a,b]	0.001
Cytochalasin E (10 μM)	150 ± 5[a]	87 ± 4[c,h]	0.001
Phloretin (100 μM)	146 ± 5[b,d]	101 ± 2[d,f,h]	0.001
Cytochalasin B (10 μM)	145 ± 5[a,c]	135 ± 5[c,d,e,g]	0.01
Phloretin (100 μM)			
Cytochalasin B (10 μM)	153 ± 5[a,b,c]	157 ± 4[a,e,f,g]	NS

Results are means ± SEM of Mg^{2+} efflux in μmol/(L cells × h) from six different experiments. 10 μM cytochalasins B and E and 100 μM phloretin were used in this study.
*Effect of cytochalasins and phloretin in glucose-free medium (rows): difference from control significant at $P < 0.01$ (a) or $P < 0.05$ (b); two by two mean difference significant at $P < 0.01$ (c,d).
†Effect of cytochalasins and phloretin medium containing 10 mM glucose (rows): differences from control significant at $P < 0.001$ (a) or $P < 0.01$ (b); two by two mean differences significant at $P < 0.001$ (c,d,e,f), $P < 0.01$ (g), and $P < 0.05$ (h).
‡Significant level of differences between means (effects of glucose).
PBS–phosphate buffered saline. NS–non significant.

completely disappeared in presence of both these compounds. This suppression of the activity of glucose on Mg^{2+} efflux suggested that it is associated with the inhibition of glucose transport. Moreover, the effects of cytochalasin B and phloretin given separately or together are significantly different from each other ($P < 0.001$). In glucose-free medium, all the compounds increased slightly (+4% to +10%) but significantly the Mg^{2+} efflux. Because this increase is observed with cytochalasin B as well as with cytochalasin E, it is unlikely to be related to the GLUT-1 transporter. At present, we have no data that allow us to explain this phenomenon.

In conclusion, we report here that glucose, which is a physiologic component of blood, partly inhibits the Na^+-independent Mg^{2+} efflux from rat erythrocytes, which is an ATP-independent process. The inhibitory activity of glucose on Mg^{2+} efflux also was observed with some other hexoses, such as mannose and fructose. The results of structure-activity relationship studies suggest the possible link between hexose transport and Mg^{2+} efflux. Moreover, the loss of glucose activity on the Mg^{2+} efflux when the glucose transport is blocked suggests that glucose carrier GLUT-1 might be involved in Mg^{2+} homeostasis. These findings must be confirmed first on nucleated cells. Second, in vivo comparative studies using strains of mice with genetically low and high erythrocyte and plasma Mg^{2+} contents[29] should provide some information that could contribute to the understanding of Mg^{2+} transport inhibition mediated by glucose.

References

1 Walker, G.M. and Duffus, J.H. (1983). Magnesium as fundamental regulator of the cell cycle. *Magnesium* **2,** 1–16
2 Quamme, G.A. (1993). Magnesium homeostasis and renal magnesium handling. *Miner. Electrolyte Metab.* **19,** 218–225
3 Heaton, F.W. (1993). Distribution and function of magnesium within the cell. In *Magnesium and Cell* (N.J. Birch, ed.), pp. 121–136, Academic Press, London, UK
4 Aikawa, J.K. (1978). Biochemistry and physiology of Mg. *Wld. Rev. Nutr. Diet.* **28,** 112–142
5 Günter, T. (1981). Biochemistry and pathobiochemistry of Mg. *Magnesium Bull.* **3,** 91–101
6 Flatman, P.W. (1993). The role in regulating ion transport. In *Magnesium and Cell* (N.J. Birch, ed.), pp. 197–216, Academic Press, London, UK
7 Walker, G.M. (1986). Magnesium and cell cycle control: An uptake. *Magnesium* **5,** 9–23
8 Wacker, W.E.C. (1980). *Magnesium in Man.* Harvard University Press, Cambridge, MA, USA
9 Masini, A., Ceccarelli-Stanzini, D., and Muscatello U. (1983). The role of Mg ions in controlling the configurational changes in isolated rat liver mitochondria. *IRSC Med. Sci.* **11,** 166–167
10 Romani, A. and Scarpa, A. (1990). Hormonal control of Mg^{2+} transport in the heart. *Nature* **346,** 841–844
11 Günther, T. and Vormann, J. (1985). Mg^{2+} efflux is accomplished by an amiloride-sensitive Na^+/Mg^{2+} antiport. *Biochem. Biophys. Res. Commun.* **130,** 540–545
12 Féray, J.C. and Garay, R. (1986). An Na^+-stimulated Mg^{2+}-transport system in human red blood cells. *Biochim. Biophys. Acta* **856,** 76–84
13 Féray, J.C. and Garay, R. (1987). A one-to-one Mg^{2+}: Mn^{2+} exchange in rat erythrocytes. *J. Biol. Chem.* **262,** 5763–5768
14 Günther, T. and Vormann, J. (1990). Characterisation of Na^+- independent Mg^{2+} efflux from erythrocytes. *FEBS Lett.* **271,** 149–151
15 Henrotte, J.G. and Franck, G. (1990). Plasma factor(s) inhibiting cell magnesium efflux determined with a biological assay rapidly. *Clin. Chem.* **36,** 1859–1860
16 Henrotte, J.G., Franck, G., and Santarromana, M. (1990). A plasma factor inhibiting erythrocyte magnesium efflux. *Magnesium Res.* **3,** 187–191
17 Henrotte, J.G., Franck, G., Santarromana, M., Bakala, J., and Lenfant, M. (1991). Inhibition of cell magnesium efflux by low molecular weight component(s) from bovine serum. *Magnesium Res.* **4,** 236
18 Stending-Lindberg, G., Penciner, J., Rudy, N., and Wacker, W.E.C. (1984). Comparison of diluents for serum magnesium estimation by atomic absorption spectrophotometry. *Magnesium* **3,** 50–56
19 Henrotte, J.G. (1980). The variability of human red blood cell magnesium level according to HLA groups. *Tissue Antigens* **15,** 419–430
20 Günther, T. and Vormann, J. (1989). Characterization of Mg^{2+} efflux in human, rat and chicken erythrocytes. *FEBS Lett.* **250,** 633–637
21 Lefevre, P.G. and Marshall, J.K. (1958). Conformational specificity in a biological sugar transport system. *Am. J. Physiol.* **194,** 333–337
22 Barnett, J.E.G., Holman, G.D., and Murday, K.A. (1973). Structural requirements for binding to the sugar-transport system of the human erythrocyte. *Biochem. J.* **131,** 211–221
23 Baly, D.L. and Horuk, R. (1988). The biology and biochemistry of the glucose transport. *Biochim. Biophys. Acta* **947,** 571–590
24 Baldwin, S.A. (1992). Mechanisms of active and passive transport in a family of homologous sugar transporters found in both prokaryotes and eukaryotes. In *Molecular Aspect of Transport Proteins* (J.J.H.H.M. DePont, ed.), pp. 169–217. Elsevier Science Publishers B.V., Amsterdam, Netherlands
25 Krupta, R.M. and Devés, R. (1986). Looking for probes of gated channels: Studies of the inhibition of glucose and choline transport in erythrocytes. *Biochem. Cell. Biol.* **64,** 1099–1107
26 LeFevre, P.G. and Marshal, J.K. (1959). The attachment of phloretin and analogues to human erythrocytes in connection with inhibition of sugar transport. *J. Biol. Chem.* **234,** 3022–3026
27 Jung, C.Y. and Rampal, A.L. (1977). Cytochalasin B binding sites and glucose transport carrier in human erythrocyte ghosts. *J. Biol. Chem.* **252,** 5456–5463
28 King, A.P.J., Tai, P.K.K., and Su, C.C. (1991). Cytochalasin B interferes with conformational changes of the human erythrocytes glucose transporter induced by internal and external sugar binding. *Biochemistry* **30,** 546–553
29 Henrotte, J.G. and Franck, G. (1997). Mice selected for low and high blood magnesium levels: A new model for stress studies. *Physiol. Behav.* **61,** 653–658