

Effect of magnesium deficiency on the metabolism of glycosaminoglycans in rats

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MS received 27 May 1986; revised 2 September 1986

Abstract. Magnesium deficiency in rats has significant effect on the concentration of different glycosaminoglycans in the tissues, the nature of the change being different in different tissues. Total glycosaminoglycans, chondroitin-4-sulphate + chondroitin-6-sulphate and dermatan sulphate increased in the aorta while hyaluronic acid, heparan sulphate and heparin decreased. In the liver, total glycosaminoglycans, hyaluronic acid, chondroitin-4-sulphate + 6-sulphate and heparin decreased while total glycosaminoglycans and all the glycosaminoglycan fractions increased in the heart. In the kidney, total glycosaminoglycans showed no significant alteration, hyaluronic acid and heparin decreased while chondroitin-4-sulphate + 6-sulphate increased. Activity of biosynthetic enzymes *viz.* glucosamine-o-phosphate isomerase and UDPG-dehydrogenase showed decrease in the liver. The concentration of 3'-phosphoadenosine 5'-phosphosulphate, activity of sulphate activating system and sulphotransferase were also similarly altered in the liver in magnesium deficiency.

Keywords. Magnesium deficiency; hyaluronic acid; heparan sulphate; chondroitin-4-sulphate; chondroitin-6-sulphate; dermatan sulphate; heparin; biosynthetic enzyme; sulphation.

Introduction

Magnesium is essentially an intracellular cation with important biochemical and physiological functions which include participation in energy metabolism, membrane permeability, electrolyte transport, nucleic acid metabolism, displacing Ca^{2+} from membrane receptors and binding sites of enzymes (Ebel and Gunther, 1983), developing of action potential (Bara *et al.*, 1983), hydroxylation of vitamin D (Traba *et al.*, 1983), transphosphorylation reactions mediated by insulin (Greenard, 1978; Ichiara and Murd, 1979), and coagulation processes (Sneddon, 1972). Some data are also available on Mg-lipid interrelationship (Rayssiguier 1983). It is known that the macromolecular components of inter-cellular matrix *viz.* proteoglycans (pg), glycoproteins, collagen, elastin etc. play, among other

Abbreviations used: pg, Proteoglycans; gg, glycosaminoglycans; TCA, trichloroacetic acid; HS, heparan sulphate; DS, dermatan sulphate; H, heparin; HA, hyaluronic acid; Ch-4S, chondroitin-4-sulphate; Ch-6S, chondroitin-6-sulphate; PAPS, 3' phosphoadenosine 5' phosphosulphate; LDL, low density lipoproteins; VLDL, very low density lipoproteins.

functions, a role in cell to cell interactions and in the transport of substances from and into the cell. Among these macromolecular components, pg are the most important, some of which are also known to be components of cell membranes. Since Mg^{2+} deficiency among other things, affects membrane permeability and transport of substances, it is necessary to know whether the metabolism of glycosaminoglycans (gg) (most of which are present in the tissues as pg) is affected in Mg^{2+} deficiency. No data seem to be available in this respect. In view of this, the effect of Mg^{2+} deficiency on the metabolism of gg has now been studied using rats as experimental animals.

Materials and methods

Male albino rats (Sprague-Dawley strain, weighing 80–100 g) were divided into the following groups with 15 rats in each group: (i) Control group, and (ii) experimental Mg^{2+} deficient group.

The diet had the following composition (g /100 g diet): Dextrose, 69.0; Casein (vitamin and fat free), 18.0; groundnut oil, 8.0; salt mixture, 4.0; vitamin mixture, 1.0.

Wesson's salt mixture without Mg^{2+} was used (Oser, 1965) $ZnCl_2$ and $CoCl_2 \cdot 6H_2O$ were also added to the diet at a concentration of 15.0 and 0.15 mg/kg diet, respectively. The Mg content of the diet was determined by analysis and $MgSO_4$ (AR : BDH) was added to the diet to give a dietary Mg level of 5 mg/100 g diet in the deficient group and 100 mg/100 g diet in the control group. All the chemicals used for the salt mixture were of analytical grade. Vitamin mixture used had the same composition as described earlier (Jayakumari and Kurup, 1979). The rats of group 1 were paired controls of those of group 2. The diet consumption was adjusted to be the same in the two groups. Deionized water after distillation was available to the rats ad libitum. The rats were housed individually in polypropylene cages in rooms maintained at 25°C. The duration of the experiment was 60 days. At the end of this period, the animals were deprived of food overnight, stunned by a blow at the back of the neck and killed by decapitation. Blood and tissues were removed to ice cold containers for various estimations.

Estimation of gg in the tissues

Dry defatted tissue was subjected to digestion with papain [crystalline papain (Sigma), 1/3 the dry weight of the tissue] (Laurent, 1960). The papain digest was deproteinised with trichloroacetic acid (TCA) (final concentration of TCA, 10% v/v) and the supernatant dialysed till free of TCA. Total gg was precipitated from the solution by the addition of 4–5 volumes of 95 % v/v ethanol containing 1.2% (w/v) potassium acetate. The precipitate collected by centrifugation was dissolved in a known volume of water. An aliquot of this solution was used to determine total gg by estimating uronic acid content by the modified procedure of Bitter and Muir (1982). Another aliquot of the gg solution (approximately 100 μ g uronic acid) was digested with testicular hyaluronidase (Sigma Chemicals, 100 units) in 0.25 M acetate buffer pH 5.3 containing 0.75 M NaCl for 16 h, under a layer of toluene. The undigested gg [containing heparan sulphate (HS), dermatan

sulphate (DS) and heparin (H)] was precipitated from the solution by 95% ethanol (v/v) containing potassium acetate as described above. The precipitate collected after centrifugation was washed with the ethanol solution, dissolved in a known volume of water and uronic acid estimated.

Another aliquot of the gg solution (approximately 100 μg uronic acid) was digested with chondroitinase ABC (Sigma Chemicals, 0.02 unit) in Tris-HCl buffer (0.25 M), pH 8.0 containing bovine serum albumin, sodium acetate and sodium chloride (50 mg, 2.4 g and 1.4 g, respectively/100 ml of the solution) for 16 h under toluene. The undigested gg [hyaluronic acid (HA), HS and H] was precipitated with 95% (v/v) ethanol as described above. The precipitate was dissolved in a known volume of water and the solution passed through a column of Dowex 1 \times 2 (Cl⁻ form), 0.5 \times 5 cm. The column was washed with water, eluted successively with 0.25 M, 1.7 M and 3.5 M NaCl solution. These eluates contained HA, HS and H, respectively. The uronic acid in each eluate was estimated as before. From these, the amount of HA, HS, chondroitin-4-sulphate (Ch-4S) + Chondroitin-6-sulphate (Ch-6S), DS and H were obtained.

Estimation of enzyme activities

The activity of D-glucosamine-6-phosphate isomerase [glutamine forming (EC 2.6.1.16) was estimated in the tissue according to the procedure of Pogell and Gryder (1957)]. Activity of UDPG-dehydrogenase (UDP-glucose: NAD oxidoreductase) (EC 1.1.1.22) activity was determined by the method of Strominger *et al.* (1957).

Sulphate metabolism

The concentration of 3' phosphoadenosine 5' -phosphosulphate (PAPS), and the activity of sulphate activating system (sulphate adenylyl transferase) (EC 2.7.7.4) and adenylyl sulphate kinase (EC 2.7.1.25) and that of aryl sulphotransferase (EC 2.8.2.1) of liver were estimated by the method of Van Kempen and Jansen (1973) using methyl umbelliferone. Details of the procedure have been described earlier (Sudhakran and Kurup, 1974).

Estimation of Mg²⁺ in the serum and tissues was carried out by atomic absorption spectrophotometry. The tissues were first ashed and then dissolved in dilute HCl. Glutathione in the erythrocytes was estimated according to the procedure of Lazarow *et al.* (1955). Protein in the enzyme extract was determined after TCA precipitation by the method of Lowry *et al.* (1972).

Statistical analysis was carried out by Student's 't' test (Bennet and Franklin, 1967).

Results and discussion

Magnesium deficient rats consumed less food (average value during the duration of the experiment being 8.0 \pm 1.0 g) and had lower weight gain. Liver and spleen weights were higher in these rats (table 1).

The concentration of Mg²⁺ in the serum in the rats of group 1 and 2 was 1.72 \pm 0.04 and 0.44 \pm 0.1 mEq/litre, respectively. The Mg²⁺ concentration in the

Table 1. Weight gain and organ weight in magnesium deficiency

Group	Weight gain (g/rat/60 days)	Liver weight (g)	Spleen weight (g)
Control	58.0 ± 1.2	4.87 ± 0.1	0.86 ± 0.01
Mg deficient	37.4 ± 0.64 ^a	6.17 ± 0.12 ^a	1.55 ± 0.03 ^a

Values are the mean ± SEM for 15 rats. Group 2 has been compared with group 1. ^a $p < 0.01$.

liver in these groups was 18.2 ± 0.49 and 14.5 ± 0.36 mEq/kg wet tissue. Concentration of glutathione in the erythrocytes in groups 1 and 2 were 372.5 ± 10.8 and $292 \pm 3.46 \mu \text{ mol}/100 \text{ ml}$ packed cells, respectively. The lower value for glutathione in the erythrocytes in Mg^{2+} deficient rats is in agreement with the results reported by Jeng *et al.* (1982).

Changes in the concentration of gg in different tissues (table 2)

Liver: Total gg, HA, 4S + 6S and H decreased in the liver in the Mg^{2+} deficient rats while HS and DS showed no significant alteration. The activity of some enzymes involved in the biosynthesis of precursors of gg (glucosamine-6-phosphate isomerase and UDPG dehydrogenase) showed a significant decrease (table 3). These enzymes catalyse rate limiting steps in the biosynthetic pathway of gg, glucosamine-6-phosphate isomerase being inhibited by feed back inhibition by UDP-N-acetyl glucosamine and UDPG dehydrogenase by UDP-xylose. Therefore the decrease in the activity of these enzymes may result in decreased biosynthesis of gg in the liver.

Sulphate metabolism in the liver (table 4): The concentration of PAPS, activity of sulphate activating system (which includes sulphate adenylyl transferase and adenylyl sulphate kinase) and that of sulphotransferase decreased in the liver in the Mg^{2+} deficient rats. The decrease in the concentration of PAPS is due to the decrease in the activity of sulphate activating system which generates it. Thus sulphate metabolism is decreased in Mg^{2+} deficiency. The decrease in the concentration of some of the sulphated gg in the liver may also be due to decreased sulphation. Thus the metabolism of gg in the liver is significantly altered in Mg^{2+} deficiency.

Aorta

The concentration of total gg and that of 4S + 6S and DS increased in the aorta in the Mg^{2+} deficient rats while HA, HS and H decreased. The aorta is particularly important in view of the high concentration of gg present. The gg are believed to play a role in the transport and accumulation of lipids in the arterial tissue. The presence of negatively charged carboxyl and sulphate groups in the gg enables these molecules to bind those bearing positive charges. Complex formation between lipoproteins [very low density lipoproteins (VLDL) and low density lipoproteins (LDL)] and gg has been shown in a number of *in vitro* studies (Burstein and Samaille, 1955; Bernfield *et al.*, 1960; Cornwell and Kruger, 1961; Iverius, 1972;

Table 2. Concentration of gg in different tissues in magnesium deficiency.

Tissue	Group	Total gg	HA	HS ($\mu\text{g/g}$ dry defatted tissue)	4S+6S	DS	H
Aorta	1	6180 \pm 150	640.0 \pm 16	1560 \pm 37.4	2430.0 \pm 58.3	890.0 \pm 23.1	540.0 \pm 14
	2	6767 \pm 203 ^a	385.3 \pm 8.7 ^a	1380 \pm 34.5 ^a	3406.4 \pm 92.0 ^a	1171.7 \pm 26.9 ^a	290.0 \pm 7 ^a
Liver	1	1072 \pm 25	150.0 \pm 4	230 \pm 5	320.0 \pm 8.0	140.0 \pm 3	200.0 \pm 5
	2	928 \pm 22 ^a	130.0 \pm 3 ^a	230 \pm 5	278.1 \pm 7.2 ^a	143.6 \pm 3.3	130.0 \pm 4 ^a
Heart	1	1540 \pm 35	340.0 \pm 9	360 \pm 9	480.0 \pm 12	160.0 \pm 3.5	140.0 \pm 4.1
	2	2440 \pm 60 ^a	600.0 \pm 15 ^a	530 \pm 12 ^a	784.9 \pm 20.4 ^a	193.2 \pm 5.4 ^a	268.3 \pm 7 ^a
Kidney	1	1720 \pm 50	335.0 \pm 8.0	330 \pm 8	555.0 \pm 12.8	223.0 \pm 5.4	227.0 \pm 6
	2	1705 \pm 45	287.9 \pm 7.8 ^a	356 \pm 9	606.3 \pm 14.6 ^a	209.1 \pm 5.5 ^a	186.0 \pm 5 ^a

Values are the mean \pm SEM for 6 rats. Group 2 (magnesium deficient) has been compared with group 1 (controls). ^a $P < 0.01$. ^b Between 0.01 and 0.05.

Table 3. Activity of glucosamine-6-phosphate isomerase (glutamine forming) and UDPG dehydrogenase in liver of magnesium deficient rats.

Group	Glucosamine-6-phosphate isomerase (glutamine forming)	UDPG dehydrogenase
	(μ mol of hexosamine/h/g protein)	(Units*/g protein)
Control	36.0 \pm 0.9	2280.0 \pm 52.4
Magnesium deficient	22.4 \pm 0.6 ^a	1865.5 \pm 48.5 ^a

*The amount of enzyme required to give an increase of 0.001 in absorbance/g protein.

Same notations as in a table 2.

Table 4. Sulphate metabolism in the liver of magnesium deficient rats

Groups	Concentration of PAPS	Sulphate activating system	Sulpho-transferase
	(μ mol of methyl umbelliferone sulphate formed/h/g protein)		
Control	165.0 \pm 4.1	27.9 \pm 0.7	22.0 \pm 0.6
Magnesium deficient	138.3 \pm 3.2 ^a	15.3 \pm 0.4 ^a	34.8 \pm 0.9 ^a

Same^a notation in table 2.

Srinivasan *et al.*, 1970). It has also been shown that gg lipoprotein complex can be extracted from the arterial tissue (Srinivasan *et al.*, 1972). The increase in the chondroitin sulphate isomers in the aorta in Mg²⁺ deficient rats along with increase in the VLDL + LDL cholesterol reported earlier (Jaya, P. and Kurup, P. A., unpublished results) may result in increased complex formation with resultant increase in the accumulation of lipids in this tissue. The results are in agreement with increased concentration of cholesterol observed in the aorta in Mg²⁺ deficient rats (Vitale *et al.*, 1957; Jaya, P. and Kurup, P. A., unpublished results).

Heart and Kidney

Total uronic acid and the concentration of different gg increased in the heart of rats fed Mg²⁺ deficient diet when compared to paired control rats. The concentration of total gg showed no significant alteration in the kidney in the Mg²⁺ deficient rats but HA and H decreased while 4S + 6S increased. There was no significant alteration in HS or DS.

Judging from the results of changes in gg in the aorta and heart now observed and the changes in the lipids in the serum and the tissues (Jaya, P. and Kurup, P. A., unpublished results), Mg²⁺ deficiency is seen to produce changes which are generally similar to those observed in atheromatous rats (Vijayakumar and Kurup,

1975; Vijayakumar *et al.*, 1975) and also in rats in which myocardial infarction is induced by isoproterenol (Mathew *et al.*, 1982). Decreased concentration of Mg^{2+} has been reported in the serum (Brown *et al.*, 1958) of patients of myocardial infarction. In endomyocardial fibrosis also, which is prevalent in Kerala, significantly lower concentrations of Mg^{2+} has been reported in the heart and the fibrotic tissues has been found to contain high concentrations of glycosaminoglycans (Valiathan, M. S., unpublished reports). Therefore the finding that Mg^{2+} deficiency induced higher concentration of gg in the heart is of significance.

References

- Bara, M., Guet-Bara, A. and Durlach, J. (1983) in *Magnesium Deficiency* (eds M. J. Halpern and J. Durlach) (London: Karger) p. 30.
- Bennet, C. A. and Franklin, N. L. (1967) *Statistical analysis in chemistry and chemical industry* (New York: John Wiley and Sons, Inc.).
- Bernfield, P., Misselbaum, J. S., Berkeley, B. J. and Ranson, R. W. (1960) *J. Biol. Chem.*, **235**, 2852.
- Bitter, T. and Muir, M. (1982) *Anal. Biochem.*, **4**, 330.
- Brown, F. D., McGandy, B. R., Gillie, E. and Doyle, T. J. (1958) *Lancet*, **2**, 933.
- Burstein, M. and Samaille, J. (1955) *C. R. Acad. Sci. (Paris)*, **241**, 664.
- Cornwell, D. G. and Kruger, F. A. (1961) *J. Lipid Res.*, **2**, 110.
- Ebel, H. and Gunther, T. (1983) *J. Clin. Chem. Clin. Biochem.*, **21**, 249.
- Folin, O. and Ciocalteu, V. (1927) *J. Biol. Chem.*, **73**, 627.
- Greengard, P. (1978) (New York: Raven Press).
- Ichihara, K. and Murad, F. (1979) *Arch. Biochem. Biophys.*, **194**, 292.
- Iverius, P. H. (1972) *J. Biol. Chem.*, **247**, 2607.
- Jayakumari, N. and Kurup, P. A. (1979) *Atherosclerosis*, **33**, 41.
- Jeng, M. HSU, Rubenstein, B. and Paleker, A. G. (1982) *J. Nutr.*, **112**, 488.
- Laurent, T. (1960) *Methods Biochem. Anal.*, **8**, 154.
- Lazarow, A. and Patterson, J. W. (1955) *Methods Biochem. Anal.*, **2**, 273.
- Lowry, D. H., Rosebrough, N. S., Farr, A. L. and Randall, R. J. (1972) *J. Biol. Chem.*, **193**, 265.
- Mathew, S., Menon, P. V. G. and Kurup, P. A. (1982) *Indian J. Biochem. Biophys.*, **19**, 352.
- Oser, B. L. (1965) *Hawk's Physiological Chemistry* (New York: McGraw Hill Book Company) p. 1377.
- Pogell, B. M. and Gryder, R. M. (1957) *J. Biol. Chem.*, **228**, 701.
- Rayssiguier, Y. (1983) in *Magnesium Deficiency* (eds M. J. Halpern and J. Durlach) (London: Karger) p. 122.
- Roy, A. B. (1971) *Biochem. J.*, **53**, 12.
- Sneddon, J. M. (1972) *Nature (London)*, **236**, 103.
- Srinivasan, S. R., Lopez, S. A., Radhakrishnamoorthy, B. and Berenson, G. S. (1970) *Atherosclerosis*, **12**, 321.
- Srinivasan, S. R., Dolan, P., Radhakrishnamoorthy, B. and Berenson, G. S. (1972) *Atherosclerosis*, **16**, 95.
- Strominger, J. L., Maxwell, R. S., Axelrod, J. and Kalekar, H. M. (1957) *J. Biol. Chem.*, **79**, 224.
- Sudhakaran, P. R. and Kurup, P. A. (1974) *J. Nutr.*, **104**, 871.
- Traba, M. L., Dela Piedra, C., Marin, A., Babe, M. and Rapado, A. (1983) in *Magnesium Deficiency* (eds M. J. Halpern and J. Durlach) (London: Karger) p. 227.
- Vankampen G. M. T. and Jansen C. S. I. M. (1973) *Anal. Biochem.*, **51**, 324.
- Vijayakumar, S. T. and Kurup, P. A. (1975) *Atherosclerosis*, **21**, 245.
- Vijayakumar, S. T., Leelamma, S. and Kurup, P. A. (1975) *Atherosclerosis*, **21**, 1.
- Vitale, J. J., White, P. L., Nakamura, M., Hegsted, D. M., Zamcheck, N. and Hellerstein, E. E. (1957) *J. Exp. Med.*, **106**, 757.