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The Postmortem Diagnosis of Magnesium Deficiency: Studies in an Animal Model for the Human Infant

Magnesium deficiency is a cause of sudden death in animals [*1–5*]. Normal-appearing mice experience violent death [*3*], while cows may die quickly and quietly [*4*].[2] The lower the age and weight of the animal when placed on the magnesium-deficient diet, the earlier and more violent the death [*2*]. Early death may follow a period of rapid growth, particularly if the diet supplies large amounts of protein, calcium, and phosphorus [*5*].

A hypothesis has been proposed that magnesium deficiency is a cause of the sudden infant death syndrome (SIDS) [*6*]. Since this death is sudden and unexpected, the cause of death would have to be established by postmortem studies.

Blood plasma values are often decreased in magnesium deficiency, but this fraction is labile, increasing precipitously in the magnesium-deficient cow at the point of death (Footnote 2). Significant increases in serum magnesium concentrations have been reported in children after death [*7*] and in normal rats that were killed with ether [*8*].

Several investigators have determined the magnesium concentration in vitreous humor [*9–12*] and liver [*13*] in SIDS victims and controls and have found no significant differences. No studies of the vitreous humor in any mammal with biochemically established magnesium depletion could be found.

The present study was conducted primarily to find the optimal tissue and conditions needed to assess the magnesium status of the weanling rat after death in order to serve as a model for the deceased human infant. Extremes of dietary magnesium were fed to normal rats and the cation composition of selected tissues was determined. The control rats were fed at least 2½ times the recommended magnesium dose of 40 mg/100 g dry diet [*1*]. The deficient animals were studied when spontaneous death from magnesium deficiency was imminent.

Materials and Methods

Animals and Animal Diets

Normal male (except when otherwise stated) weanling Sprague-Dawley rats weighing 28 to 40 g were purchased from Hilltop Farms, Scottdale, Pa. Their care and diets were as previously described [*5*], with few modifications. The purified diet contained 0.3 mg of magnesium per 100 g of dry diet, to which was added 0 to 150 mg of magnesium per 100 g of dry diet. Diets for the one-week study of acute magnesium deficiency contained 20% casein; those for the two-week study of attenuated magnesium deficiency contained

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40% casein. The natural control, Ralston laboratory chow 5001, was fed for one or two weeks (Table 1).

Procedure

Rats with no known previous seizures were anesthetized with 5 mg of sodium pentobarbital per 100 g body weight intraperitoneally, and blood was drawn percutaneously from the heart. These specimens were analyzed for plasma cations [*5*]. Rats were then killed with an overdose of sodium pentobarbital, and vitreous humor was promptly aspirated with a #19 gage needle. Vitreous humor samples from two or more rats were pooled, dried, and ashed without defatting. The liver, heart, skeletal muscle (m. quadriceps femoris), entire sternum, entire femur, and left fifth and sixth ribs (combined into one sample) were dissected, dried, defatted, and homogenized; duplicate specimens were prepared for analysis according to the method of MacIntyre and Davidsson [*14*], with few modifications. Dilutions were made in appropriate solutions of lanthanum chloride, and magnesium, calcium, potassium, and sodium were determined on a Model 303 Perkin-Elmer atomic absorption spectrophotometer. Statistical analyses were made by using standard tables for two-tailed Student's t distribution [*15*].

Sex differences were studied by comparing the cation values in soft tissues and bones from groups of six male and six female rats fed various diets. Distribution of magnesium within bones was determined by comparing the values for combined anterior and posterior rib halves from nine control and eight deficient rats. Finally, a serial study of the magnesium concentration of plasma, the sternum, and the femur was made in rats on Days 0, 3, and 6.

Results

The growth records of ten rats in the five diet groups are shown in Fig. 1. After an initial adjustment period for the rats fed laboratory chow (LC), there was no significant difference in the growth rates between the three groups of control animals. The controls approximately doubled their initial weight at one week and tripled it at two weeks. For the first four days, the 0-20 rats grew as well as the controls, but then they became anorexic, with reduction in the growth rate.

Figures 2, 3, and 4 show the magnesium concentrations of selected tissues from rats fed the purified diets. Plasma values were expressed in meq/litre; vitreous humor, in meq/kg dry weight (DW); and all other tissues, in meq/kg dry fat-free weight (DFFW). Each symbol in Figs. 2, 3, and 4 represents the magnesium concentration from one sample. All control values are represented by solid triangles, and values from magnesium-deficient rats, by hollow circles. In the text that follows, values are given as the mean plus

TABLE 1—*Magnesium and protein content in experimental diets.*

Clinical Status of Animals	Duration of Study, weeks	Magnesium, mg/100 g	Protein, %	Source of Protein	Designation of Diets
Purified control	1	100	20	casein	100-20
Acute magnesium deficiency	1	0	20	casein	0-20
Purified control	2	150	40	casein	150-40
Attenuated magnesium deficiency	2	5	40	casein	5-40
Natural control, laboratory chow 5001	1 or 2	210	23.4	crude vegetable	LC

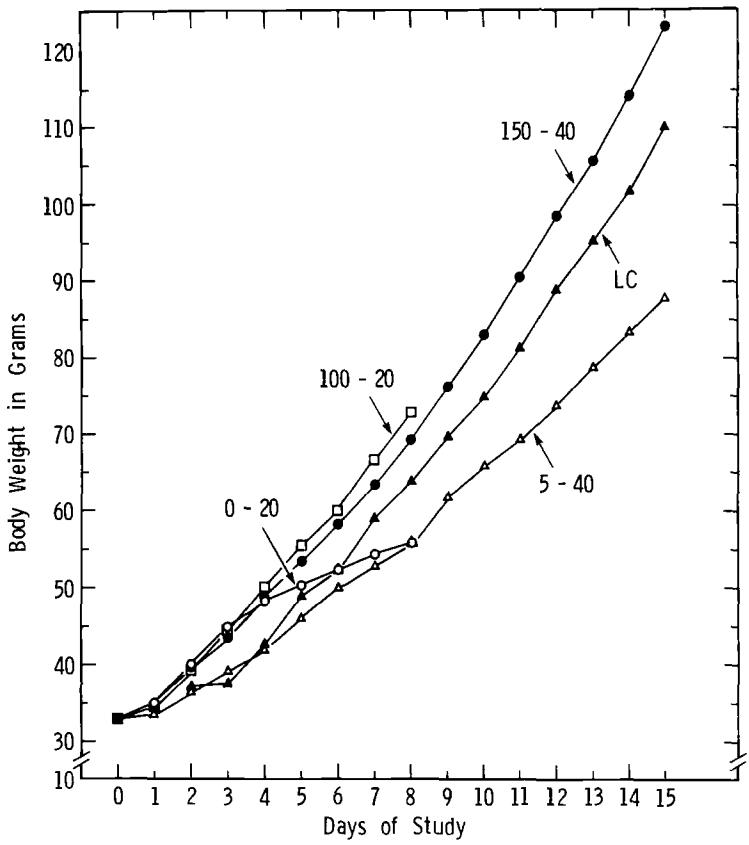

FIG. 1—*Growth records in ten rats in a group fed laboratory chow (LC) and in four groups fed purified diets that were identified numerically, the first number representing magnesium in mg/100 g and the second number the percentage of casein: control diets were 100–20 and 150–40, and deficient diets were 0–20 and 5–40. Note that after an initial adjustment period, the control rats gained weight equally well, while weight gain in the 0–20 rats decreased after Day 4.*

or minus the standard deviation (SD); the numbers in parentheses indicate the number of samples making up the mean.

Figure 2 shows the antemortem magnesium concentration in blood plasma from control and magnesium-deficient rats at one and two weeks of study. Note that a wide disparity was found in values from control and magnesium-deficient animals, with no overlapping of individual values between the two groups.

Figure 3 contains values for the magnesium concentration in tissues from control rats fed the 100-20 diet and the acutely deficient rats fed the 0-20 diet. A significant difference ($P < 0.01$) was found in the vitreous humor between magnesium deficient and control animals fed the 100-20 diet, but the range was small, and there was much overlapping of individual values between the two groups. The magnesium concentration of vitreous humor from eleven laboratory chow-fed rats (three samples) was 52.8 ± 2.9, a value between the 100-20 and 0-20 rats, and not significantly different from either.

Although the mean control value for liver, 67.3 ± 4.4 (13), was significantly different from that of the acutely deficient rats, 72.3 ± 4.6 (19) ($P < 0.01$), there was much overlapping of individual values between the 100-20 control and 0-20 deficient animals.

The magnesium concentrations of the myocardium and skeletal muscle also fell within narrow ranges, with overlapping of individual values between the control and deficient

groups. For both of these tissues, the difference in means was significant at the level of $P < 0.001$. However, no significant difference was found when mean values for skeletal muscle from the deficient 0-20 rats, 100.8 ± 3.9 (56), were compared with control values from laboratory chow-fed rats, 103.7 ± 4.7 (5).

The magnesium concentrations of sternum, femur, and rib are presented in the last three columns of Fig. 3. Note the variation in bones. The mean control value of the sternum, 143.6 ± 11.0 (23), was significantly different from that of the femur, 268.7 ±

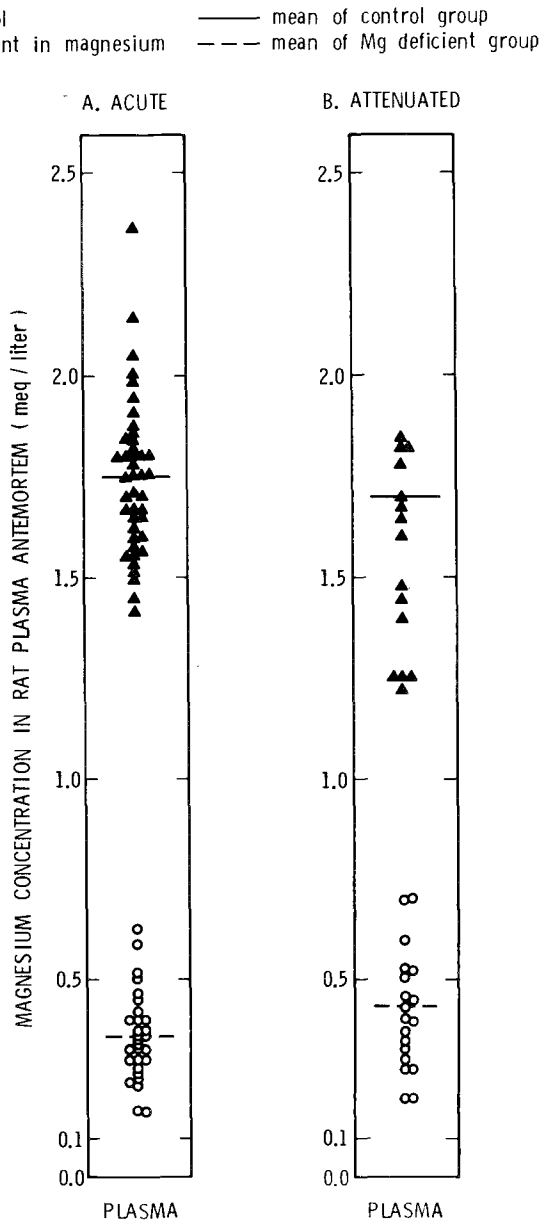

FIG. 2—*Magnesium concentrations in blood plasma from the magnesium-deficient rats in the acute and attenuated experiments, studied antemortem. These rats had had no known seizures. Values from the deficient rats were significantly different from the controls* (P < *0.001*).

26.6 (54) ($P < 0.001$), and from that of the rib, 261.5 ± 13.9 (5) ($P < 0.001$); the rib was not significantly different from the femur. The mean magnesium value in the sternum of the 0–20 magnesium-deficient rats was 69.9 ± 8.2 (58), approximately half of the control value ($P < 0.001$), while that of the femur, 98.0 ± 17.8 (107), and the rib, 102.7 ± 10.3 (5), were about 40% of the control values ($P < 0.001$).

Figure 4 shows the magnesium concentration in tissues from rats with attenuated magnesium deficiency and their controls studied at two weeks. In general, these data were similar to that from the acutely deficient animals (Fig. 3), except that the differences in the magnesium concentration of the heart and the skeletal muscle between control and deficient animals were not significantly different. Again, the mean for the sternum and femur were strikingly different ($P < 0.001$), and there was no overlapping of individual values.

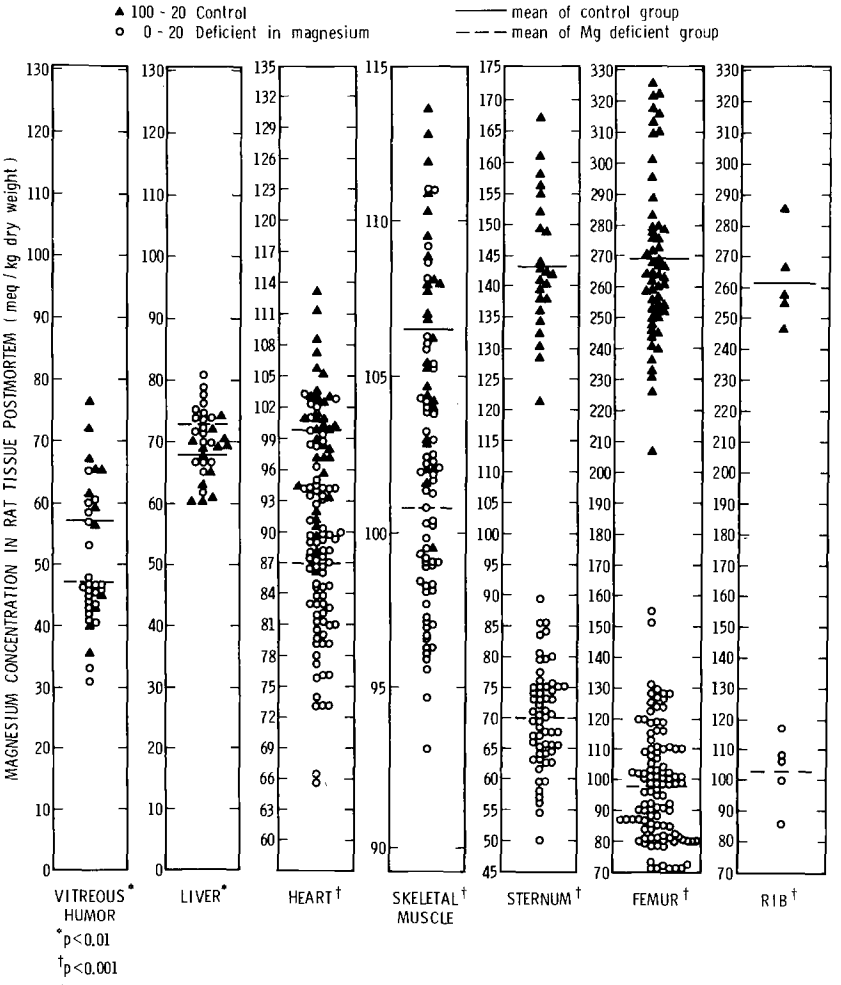

FIG. 3—*Magnesium concentrations in selected tissues from weanling control rats and rats with acute magnesium deficiency. Values for magnesium in vitreous humor were expressed as meq/kg dry weight, while values for other tissues were in meq/kg dry fat-free weight.*

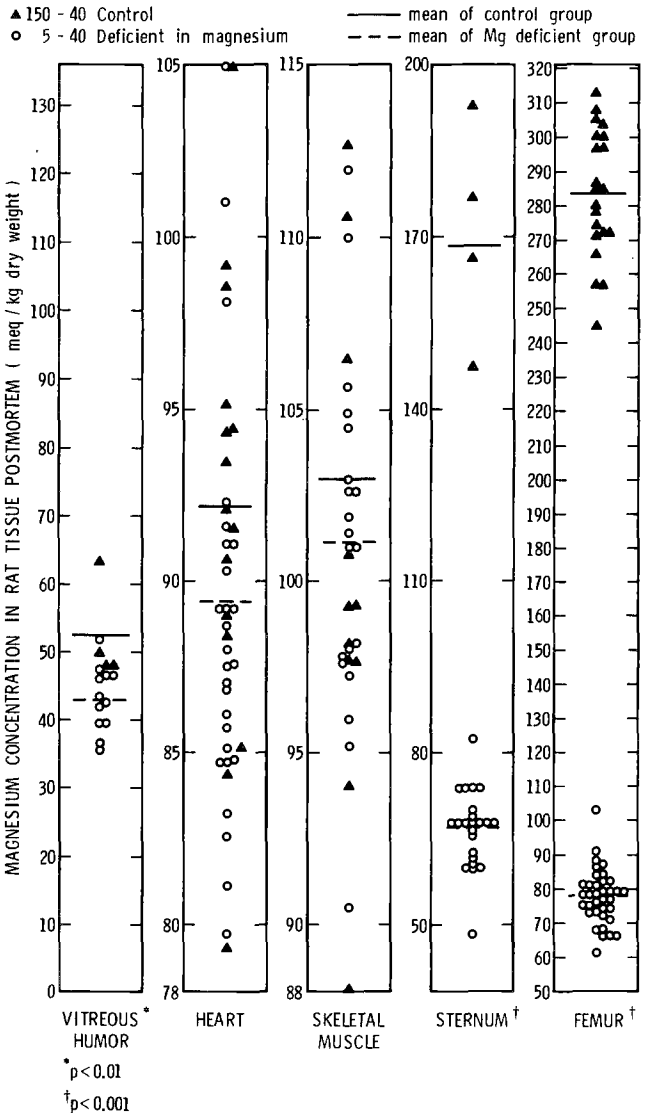

FIG. 4—*Magnesium concentrations in selected tissues from weanling control rats and rats with attenuated magnesium deficiency. The units were as in Fig. 3.*

Changes in Other Cations Secondary to Magnesium Deficiency

Table 2 contains the calcium, potassium, and sodium concentrations found in selected tissues from 100-20 controls and 0-20 deficient rats. Changes inconstantly found in the magnesium-deficient animals included increased calcium and sodium and decreased potassium.

A similar pattern of change was found in a study of attenuated magnesium deficiency, comparing values from 150-40 controls and 5-40 rats at two weeks. In addition to the above changes, there was a decrease in the potassium value in skeletal muscle, from 304.0 ± 34.2 (12) in the controls to 271.0 ± 34.5 (20) in the deficient rats. The difference was significant at $P < 0.02$.

TABLE 2—Cation values in 100-20 control and 0-20 rats with acute magnesium deficiency.

Tissues and Units	Number	Calcium	Potassium	Sodium
Vitreous humor, meq/kg dry weight				
100-20	12	15.21 ± 5.48[a]	157.7 ± 54.3	812.5 ± 216.5
0-20	23	17.35 ± 4.82	154.4 ± 46.2	797.2 ± 288.5
P[b]	...	NS[c]	NS	NS
Liver, meq/kg DFFW[d]				
100-20	13	5.61 ± 0.74	293.4 ± 25.8	129.3 ± 22.8
0-20	19	9.95 ± 2.58	293.8 ± 40.1	139.9 ± 26.4
P	...	0.001	NS	NS
Heart, meq/kg DFFW				
100-20	36	6.34 ± 0.96	240.2 ± 42.5	203.0 ± 50.9
0-20	77	7.96 ± 1.38	241.9 ± 46.7	201.1 ± 56.2
P	...	0.001	NS	NS
Skeletal muscle, meq/kg DFFW				
100-20	26	8.32 ± 0.78	284.1 ± 43.3	152.4 ± 28.4
0-20	56	11.74 ± 2.92	295.0 ± 56.1	160.0 ± 26.2
P	...	0.001	NS	0.05
Sternum, meq/kg DFFW				
100-20	23	2580 ± 334	186.5 ± 11.0	195.8 ± 26.7
0-20	58	2682 ± 357	184.7 ± 15.6	210.3 ± 26.3
P	...	0.001	NS	0.025
Femur, meq/kg DFFW				
100-20	53	6736 ± 614	170.0 ± 16.4	244.0 ± 20.8
0-20	106	7060 ± 613	158.4 ± 16.5	266.7 ± 31.1
P	...	0.005	0.001	0.001

[a] Mean ± SD.
[b] Standard probability value when compared with 100-20 control by two-tailed Student's *t* test.
[c] NS = not significantly different.
[d] DFFW = dry fat-free weight.

No significant differences in cation values were seen when tissues from six male and six female rats fed 100-20 diet, or from similar groups of animals fed 0-20 diet, were compared.

Distribution of Magnesium in the Rib

The study of the anterior and posterior halves of ribs from nine 100-20 control rats revealed a significant difference ($P < 0.05$), with the higher values in the posterior halves, 343.6 ± 12.6, compared to 329.0 ± 17.9 in the anterior halves.

The study of the anterior and posterior halves of ribs from eight 0-20 rats acutely deficient in magnesium revealed a greater difference, $P < 0.001$. Again, the higher magnesium concentration was in the posterior halves: 134.1 ± 8.8 compared to 112.8 ± 8.9 in the anterior halves.

Effect of the Duration of Magnesium Deficiency

A rapid decrease in magnesium concentration in plasma, sternum, and femur was found when the values found on Day 0 were compared with those of Day 3 and those of Day 3 were compared with the concentrations found on Day 6, for the 0-20 rats. The *P* value for each was <0.001. The plasma values were 1.93 ± 0.23 (3) on admission, 0.44 ± 0.06 (4) on Day 3, and 0.29 ± 0.04 (15) on Day 6. Corresponding values in the sternum from the same animals were 161.5 ± 2.4 (4) on admission, 93.1 ± 5.5 (4) on Day 3, and 74.5 ± 8.5 (15) on Day 6. The magnesium concentrations in the femur in those rats

decreased from 278.3 ± 11.2 (4) on admission to 133.8 ± 11.9 (4) on Day 3 and to 94.1 ± 8.4 (15) on Day 6.

Discussion

The literature regarding changes in cations in mammalian tissues in magnesium deficiency reveals conflicting results [*16*], probably explained by differences in species, age, dietary components, duration of the study, and the rate of growth. The present study is unique in that the rats were models for the human infant of several months to one year of age in terms of time span and in terms of the increase in body weight during the study period. The age was chosen because the sudden infant death syndrome (SIDS or crib death) usually occurs during the first year of life, with a peak incidence between two and four months [*17*]. The rat is premature at birth by human standards, and three years in the life of a rat is approximately equal to 90 years of human life [*18*]. Therefore one week in the life of a newly weaned rat would approximately equal six months of human life. The control rats in the present study doubled their weight by the end of the first week and tripled it by the end of the second week. The full-term human infant will generally double his birth weight by five months and triple it in one year [*19*].

We measured the cation composition in tissues in animals fed diets that either provided an abundance of magnesium or that resulted in lethal magnesium deficiency. Of course it would not be possible to find groups of human infants fed such extreme differences in magnesium. Therefore only tissue that showed distinctly different magnesium concentrations under the extreme conditions of this study should be considered suitable to diagnose magnesium deficiency.

Within well-defined limits, vitreous humor is a valuable diagnostic tissue. Coe [*20*] found that it accurately reflected certain antemortem changes in sodium, chloride, urea nitrogen, and glucose. Swift and associates [*21*] studied the vitreous magnesium in infants postmortem and found that the vitreous magnesium levels were higher than the generally accepted normal levels for plasma magnesium; that the vitreous magnesium levels were age-related, with highest values in the neonate; and that there was a considerable range of values for each age group. No correlation was made between the vitreous magnesium values and the magnesium values of other tissues. In the present study, the vitreous humor did not accurately reflect the degree of magnesium deficiency.

It is generally agreed that the concentration of magnesium in the liver does not reflect the body stores of magnesium [*22*]. Schwartz and associates [*23*] found the liver to be minimally affected by the mineral content of the diet and postulated that magnesium may be preferentially preserved in the cellular sites of active protein synthesis. Martindale and Heaton [*24*] noted that the liver behaved differently from other organs studied during magnesium deficiency and that the magnesium content of the liver rose 18% during the first 15 days of deficiency. In the present study, the magnesium concentration in the liver of acutely deficient animals was slightly higher than the control value.

The heart and skeletal muscle were both found to be unreliable guides to establish the diagnosis of magnesium deficiency in the young, rapidly growing animal.

Bone best reflected the magnesium content of the diet. It has been estimated that 70% [*25*] of the magnesium in the human body is in the skeleton. We found that the bones of the same animal did not all have the same mineral composition; one bone must be selected for study. The two most readily accessible bones at the autopsy of the human infant are the sternum and the rib. There are six ossification centers in the sternum and four in the rib [*26*]. The fact that three of the four ossification centers in the rib are in the posterior end of the rib probably explains the higher magnesium concentration in the posterior halves. Therefore, because of irregular and changing mineral distribution within a bone in the young mammal, the entire bone should be sampled, and controls and study

subjects should be carefully matched for age. The rapidity of change in the magnesium concentration in bone in the 0-20 rats in this study attests to the dynamic metabolism of magnesium in bone.

Conclusions

Vitreous humor, liver, heart, and skeletal muscle did not consistently show significant decreases in magnesium concentrations in animals with severe dietary magnesium deficiency; therefore, those tissues are considered poor choices for the postmortem diagnosis of magnesium deficiency in the young mammal.

Bone was found to be the best tissue to determine whether or not a young mammal has adequate magnesium stores. Since the bones in the same animal did not all have the same mineral concentration, one bone must be analyzed. The entire sternum or the entire rib would be appropriate choices for the human infant studied after death. These data demonstrate the sensitivity with which bone reflected the known level of dietary magnesium and the clinical status of the animal. The need to match control and study subjects for age was apparent.

Summary

Weanling rats were studied as a model for the human infant to determine the optimal tissue in which to assess the status of magnesium after death. Control rats were fed laboratory chow or purified diets that provided a surfeit of magnesium and accommodated a normal rate of growth. Other rats were fed diets that resulted in two degrees of magnesium deficiency: one that might result in spontaneous death within one week, and the other, within two weeks. These times may correlate with six months and one year in the human infant, the period during which the sudden infant death syndrome usually occurs.

There was no consistent difference between the magnesium concentration found in the vitreous humor, liver, heart, or skeletal muscle of magnesium-deficient and control rats. However, bone accurately reflected the level of dietary magnesium. There was a significant difference between the magnesium concentration of the anterior and posterior halves of the ribs, indicating irregular distribution of magnesium within the bone. Significant differences were found in the magnesium concentrations of different bones from the same animals. Therefore one entire bone, such as the sternum or the rib, should be studied. The need to match control and study subjects for age was apparent.

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