Magnesium Deficiency in the Cebus Monkey

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Various aspects of dietary magnesium deficiency have been studied extensively in the rat,1,2 and guinea pig3 and to a lesser extent in other species.4 During the last few years there has been an increased interest in magnesium metabolism as evidenced by the number of papers in the literature dealing with this ion in various clinical states. Alterations in magnesium metabolism have been reported to occur in a variety of diseases; particularly in diseases associated with the thyroid, in alcoholism, in cirrhosis, in pregnancy, in newborn infants and in certain patients with renal and cardiac disease.4,9

In our own laboratory we have been interested in the effects of magnesium deficiency on cardiac metabolism and on the production of atherosclerosis. High dietary magnesium has been shown to diminish the amount of cardiovascular lipidosis in rats fed cholesterol and cholic acid. Conversely, magnesium deficiency enhances lipid deposition within the left ventricular valves and aorta.10-12 In a recent study magnesium deficiency in young growing puppies fed no cholesterol or cholic acid resulted in the production of cardiac arrhythmias, cardiovascular lesions, intimal proliferation and a marked sensitivity to cardiac glycosides.13

The present study deals with the effect of magnesium deficiency on weight gain, serum electrolytes, serum cholesterol, histopathology, the electrocardiogram and the tolerance to cardiac glycosides in the Cebus (fatuella) monkey.

Methods

Young growing male monkeys (Cebus fatuella) weighing approximately one kilogram were divided into two groups of eight. The control group was fed a diet of the following constituents per 100 grams of food: casein (purified), 10; glucose, 72.8; corn oil, 9.0; cod liver oil, 1.0; salt mixture, 2.5; choline chloride, 0.3; cholesterol, 1.0; and calcium carbonate CaCO3, 1.5. The salt mixture used was that of Jones and Foster14 with the MgSO4 and CaCO3 removed. Magnesium was added to the control diet at a level of 96 milligrams per 100 grams of diet as the magnesium oxide replacing an equivalent amount of glucose. The following amounts of vitamins expressed in milligrams were added per kilogram of diet: thiamine hydrochloride, 4; riboflavin, 8; pyridoxine hydrochloride, 4; niacinamide, 40; calcium pantothenate, 20; folie acid, 1; and biotin, 0.2. The deficient group was fed the same diet but one containing only six milligrams of magnesium per hundred grams. The diet, as well as water, was fed ad libitum.

The animals were housed in individual cages with screened bottoms. Venous blood was drawn approximately every two weeks and the serum analyzed for sodium and potassium by flame photometry. The following blood and serum constituents were also determined: serum cholesterol,16 serum protein,17 hemoglobin,17 hematocrit by a micro capillary method and serum magnesium.18 At monthly intervals electrocardiograms were taken on monkeys anesthetized with sodium pentobarbital (30 mg per kilogram, iv).

At the end of 190 to 210 days animals from both the control and deficient groups were anesthetized with sodium pentobarbital (30 mg per kilogram, iv). At the end of 190 to 210 days animals from both the control and deficient groups were anesthetized with sodium pentobarbital (30 mg per kilogram, iv) and their sensitivity to a cardiac glycoside (K-strophanthinid) studied. The method of administering the cardiac glycoside was as follows: 0.05 milligrams of K-strophanthinid was given intravenously through an indwelling catheter (femoral vein) over a five-minute period. Thereafter, 0.1 milligram of the cardiac glycoside in 0.5 ml of saline was administered every five minutes.

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FIGURE 1

Electrocardiograms (Lead II) of control (A) and magnesium deficient monkeys (B). Note the changes in the T-wave (peaking) and ST segment due to magnesium deficiency.

The electrocardiographic, arterial and venous pressure changes were recorded before and during the experiment. The end-point was four consecutive ventricular premature contractions. The limb leads, unipolar leads and a right and left unipolar precordial lead were recorded. After the end-point had been reached, the animals were sacrificed and portions of various organs were fixed in neutral buffered 10% formalin and examined microscopically. A variety of stains was used. The heart was opened through the left ventricle in order to expose the left ventricular valves, ascending and descending aorta. This preparation after formalin fixation was stained with Sudan IV as previously described.

Results

During the course of the experiment the control animals gained an average of 260 grams whereas deficient animals lost, on average, approximately 40 grams. It is clear from table 1 that in the animals fed the control diet, little change occurred in the various blood and serum constituents measured from initial values. As was expected, the magnesium deficient animals showed a significant decrease in the concentration of serum magnesium from an initial value of approximately 1.3 to 0.7 milligrams per cent prior to sacrifice. In addition, the serum potassium decreased significantly from 6 mEq/L to 4 mEq/L in deficient animals. The only other change seen was in the serum cholesterol.

| TABLE 1 |
The Effect of Magnesium Deficiency on Various Blood Components of the Cebus Monkey

| Component       | Control*       | Deficient      | P < |<0.05
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<tr>
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<tr>
<td>Hemoglobin (gm %)</td>
<td>12.4 ± 1.71</td>
<td>13.8 ± 1.5</td>
<td></td>
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<tr>
<td>Hematocrit (%)</td>
<td>49 ± 1.0</td>
<td>40 ± 1.0</td>
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<tr>
<td>Serum Na&quot; (mEq/L)</td>
<td>5.2 ± 0.8</td>
<td>6.3 ± 0.4</td>
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<tr>
<td>Serum Mg&quot; (mg %)</td>
<td>1.50 ± 0.31</td>
<td>1.32 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Serum protein (g %)</td>
<td>6.9 ± 0.4</td>
<td>6.7 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol (mg %)</td>
<td>138 ± 18</td>
<td>137 ± 17</td>
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</tbody>
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*Animals fed experimental diets for 180-210 days before sacrifice. Eight monkeys per group.
†Probability (<0.05 = statistical significance).
‡Standard error of the mean.
level which rose significantly in the deficient group from 170 to approximately 300 milligrams per cent.

Figure 1 illustrates some of the changes seen in the electrocardiograms of magnesium deficient monkeys. Normally these monkeys have a heart rate of approximately 250 per minute. However, significant changes in the rate and in the electrocardiogram were observed in magnesium deficient animals. The rate was usually reduced one-third to one-half of the original rate in severely deficient monkeys. The main electrocardiographic changes consisted of an elevated ST segment and a marked peaking of the T wave. In some animals there occurred a depression of the ST segment although this was infrequent. These changes in the ST segment and T wave are consistent with changes seen in hyperkalemia. However, as shown in table 1, the deficient animals actually developed a hypokalemia.

In addition to a decreased tolerance by magnesium deficient monkeys to cardiac glycoside administration, the changes occurring in the electrocardiogram during digitalization seen in deficient animals were not seen in the control group. The average dose of K-strophanthinid necessary to induce four consecutive ventricular ectopic beats in deficient monkeys was 0.07 milligram per kilogram whereas almost twice as much, 0.11 milligrams per kilogram, was necessary to induce toxicity in control animals.

Only slight or minimal changes in the electrocardiogram of control monkeys were
FIGURE 3

(A) Sudanophilic deposits in ascending aorta of magnesium deficient monkey. (B) Raised intimai sudanophilic deposits in descending aorta. (C) Raised plaque at center and top with sudanophilic stain on periphery.
observed during digitalization. With continued digitalization, ventricular ectopic beats became apparent. One could always distinguish deficient from control monkeys, in that the former always developed various cardiac arrhythmias during digitalization before the appearance of ectopic beats. The arrhythmias were of the nodal type and in the same animal one could see fluctuations between lower, middle and upper nodal rhythms. Figure 2A shows an upper nodal rhythm produced during digitalization of a deficient monkey; there is an inverted P in Lead II. Figure 2B illustrates middle nodal rhythm; note the absence of auricular activity (Lead II) and the presence of ventricular ectopic beats.

Control animals showed little, if any, macroscopic vascular fat (lipidosis). Deficient animals showed marked lipid deposition in both the ascending and descending aorta (fig. 3A and B). Figure 3C shows a raised fibrous intimal plaque with fat appearing only at the periphery.

Figure 4 shows a microscopic section of the aorta of a magnesium deficient monkey. Numerous fat-laden cells are seen in the intima of the artery. The accumulation of these cells produces elevation of the intimal surface. Similar cells were seen in small numbers between the laminae of elastic layers of the artery. Figure 5 illustrates one of the elevated plaques stained with hematoxylin and eosin. The intima is thickened and proliferating fibroblasts are present.

Figure 6 shows the edge of a fibrous plaque stained for elastic tissue. Elastic fibers are interrupted, fragmented in many areas and, in between the elastic laminae, fibrosis can be seen. Figure 7 shows a section of myocardium from a deficient animal stained with hematoxylin and eosin. The myocardial fibers have become deeply eosinophilic and have lost their striations and acquired a hyaline appearance. This change is limited only to a few fibers and in the same field, normal appearing fibers are observed.

The changes in the electrocardiogram and the deposition of lipid in the aorta of deficient animals were consistent findings. Control animals had little, if any, lipid deposition in their aortae and never developed significant electrocardiographic alterations.
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FIGURE 6
Elastic stain (van Gieson) of the edge of an aortic fibrous plaque. Elastic fibers are interrupted and fragmented in many areas of the intima and media.

Discussion

It is obvious that the monkey (Cebus fatuella) differs markedly from the rat, guinea pig and dog in that magnesium deficiency in this species produces vascular lesions without the deposition or presence of calcium. The rat,1-7 dog,18 mouse,8 guinea pig,9 and calf20 all demonstrate metastatic calcification of the soft tissues with magnesium deficiency. In addition, magnesium deficiency produces intrarenal calcification in rats and guinea pigs, whereas in the Cebus monkey, no calcification was seen in any of the tissues or organs studied.

Similarly, in the rat and guinea pig magnesium deficiency was without any apparent effect on the level of serum cholesterol. In the present study deficient monkeys had increased serum cholesterol concentrations. The correlation between serum magnesium and cholesterol in humans has not been established. While some investigators have found a significant inverse relationship between serum magnesium and cholesterol, others have failed to show such a correlation.22-25

Various theories concerning the pathogenesis of atherosclerosis have been offered. Some believe that, initially, fatty infiltration occurs while others feel that the fatty infiltration follows an initial arterial damage or injury.26 The results of the present and previous studies using rats would suggest that perhaps in magnesium deficiency there is a subintimal lesion as evidenced by the marked fragmentation and rupturing of the elastic fibers in the media. In the rat, medial lipidosis appears at about the same time one sees intimal lipidosis.1-13 In the monkey the rupturing and fragmentation of these elastic fibers accompanies the appearance of lipid-laden cells in the intima and media of the aorta. In some places lipid-laden cells are seen only at the periphery of a plaque composed of deposits of connective tissue and collagen fiber. Although the proliferation of connective tissue could be taken as a phenomenon separate from the fat deposition, the appearance of fat-laden cells at the periphery would favor the view that the connective tissue replaced a previous fat deposit. Thus, as the

FIGURE 7
Hematoxylin and eosin stain of the myocardium of a magnesium deficient monkey. Some of the myocardiad fibers have become deeply eosinophilic with loss of striations and acquisition of a hyalin appearance.
deficiency progresses, fat disappears and the lesion becomes fibrous in nature.

Others, however, suggest that the "metabolic role" of the aortic tissue itself may be important in predisposing to atherosclerosis. In a previous study using guinea pigs and rats fed cholesterol-containing diets, the guinea pig was more susceptible to atherosclerosis than was the rat at equally elevated serum cholesterol levels (300 to 400 mg%). Thus, it was not possible to predict the severity or the degree of atherosclerosis in one species from the serum cholesterol level in another and presumably the guinea pig aorta is more sensitive to the hypercholesterolemia produced.

The level of serum cholesterol in the magnesium deficient monkeys was not greatly elevated but these animals nevertheless developed marked vascular lipidosis and atherosclerosis. One may explain the development of atherosclerosis without a markedly elevated serum cholesterol concentration if the primary lesion in magnesium deficiency is a subintimal one.

The magnesium deficient monkey, like the magnesium deficient dog, developed decreased tolerance to cardiac glycosides. It has been postulated that the decreased tolerance to cardiac glycosides is due to a decreased intracellular potassium. The preponderance of data in the available literature would indicate that hypokalemia, and presumably low intracellular potassium, renders the animal or human being sensitive to cardiac glycosides and that in most cases the administration of potassium and perhaps magnesium abolishes the cardiac arrhythmias produced by overdoses of cardiac glycosides. It has also been demonstrated that magnesium deficiency results in a secondary depletion of intracellular potassium. In the present study not only was there a decreased tolerance to cardiac glycosides in magnesium deficient animals, but such animals, in contrast to controls, always developed arrhythmias during digitalization.

The magnesium deficient monkeys showed profound changes in the electrocardiogram. These changes consisted mainly of an elevation of the ST segment and a peaking of the T wave, as shown in figure 1B, and are consistent with those seen in hyperkalemia. The serum potassium level, however, in deficient monkeys was low (table 1). The seeming paradox can best be explained by assuming a relatively greater intracellular deficit of this cation than occurred extracellularly resulting in a relative hyperkalemia. However, it still remains to be proved that, indeed, a decreased intracellular potassium in deficient monkeys occurred. This is strongly suggested by the data on the tolerance of cardiac glycosides in which deficient animals tolerated lesser amounts than controls and on the changes seen in the electrocardiograms of such animals during digitalization.

Magnesium is essential for oxidative phosphorylation with an uncoupling of this reaction in mitochondria isolated from magnesium deficient rats. Similarly, various studies have demonstrated that intact, viable mitochondria with the ability to carry out oxidative phosphorylation are required for maintaining sodium and potassium concentration gradients. Thus, the electrocardiographic changes and decreased tolerance to K-strophanthidin seen in the deficient monkeys may be the result of intracellular losses of potassium and, perhaps, of magnesium as well.

The relevance of these results to human coronary disease still remains to be demonstrated. However, Parsons, et al. reported that over 100 patients suffering from coronary heart disease (of which at least one-third were acute myocardial infarctions) were treated with intramuscular magnesium sulfate with only one death, while 196 cases of acute myocardial infarction treated with routine anticoagulants gave a 30% mortality. Malkiel-Shapiro, et al. reported similar findings.

Summary

Purified diets containing cholesterol and deficient or adequate in magnesium were fed to young growing Cebus (fatuella) monkeys.
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Diet-induced magnesium deficiency resulted in weight loss, hyperirritability, and in several monkeys, convulsions.

In addition to the expected hypomagnesemia, deficient animals also had decreased serum potassium levels. However, after several weeks on diet, the electrocardiogram of deficient animals showed significant changes consisting primarily of S-T segment depression and peaking of the T wave; changes usually seen in hyperkalemic states.

Prior to sacrificing the animals, the tolerance to K-strophanthidin, a cardiac glycoside, was ascertained in both deficient and control animals. The cardiac glycoside was administered slowly by the intravenous route to anesthetized monkeys and the appearance of four consecutive ventricular premature contractions in the electrocardiogram was taken as the end-point. Deficient animals required approximately one-half the dose administered to control monkeys to develop extrasystoles.

The histopathology consisted of marked vascular sudanophilic deposits and dense fibrous connective tissue plaques in the ascending and descending aorta of deficient monkeys. Control animals showed little, if any, vascular sudanophilia.

The serum cholesterol concentration increased significantly in the deficient group while the rise seen in control monkeys lacked statistical significance.

References


