Effects of Magnesium-Deficient Diet Upon Puppies

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MOTOUMI NAKAMURA, M.D., AND BERNARD LOWN, M.D.

CERTAIN ASPECTS of dietary magnesium deficiency have been studied extensively in the rat and less so in the guinea pig, calf, duck, and dog. Excellent reviews of magnesium metabolism and requirement have been published by Wacker and Vallee and O'Dell. Magnesium deficiency results in calcification of the heart and kidney. High dietary magnesium has been shown to diminish the amount of cardiovascular lipidosis in rats fed cholesterol and cholic acid. Conversely, magnesium deficiency enhances the lipid deposition within the left ventricular valves and aorta. Because magnesium deficiency produces profound changes in the myocardium and enhances cardiovascular sudanophilia, it was believed advisable to study magnesium deficiency in dogs prior to undertaking a study of feeding cholesterol to magnesium-deficient dogs. The dog, unlike the rat, develops arteriosclerosis spontaneously, and these lesions resemble in part those seen in the human. In addition, being relatively large, the dog lends itself to procedures such as repeated blood withdrawal, the taking of electrocardiograms, and various other physiological measurements. The present study deals with the effect of magnesium deficiency on weight gain, serum electrolytes, serum cholesterol, histopathology, and the electrocardiogram.

Methods

Experiment 1

Six male, mongrel puppies, approximately six to eight weeks old and weighing 3 to 5 Kg., were fed a diet of the following constituents per hundred grams of food: casein (purified), 20; glucose, 62.8; corn oil (Mazola), 9.0; cod-liver oil, 1.0; cellulose, 3.0; salt mixture, 6.25; choline chloride, 0.2; and CaCO3, 1.5. The salt mixture used was that of Jones and Foster with the MgSO4 and CaCO3 removed. The following amounts of vitamins expressed in milligrams were added per kilogram of diet: thiamin hydrochloride, 4; riboflavin, 8; pyridoxine hydrochloride, 4; niacinamide, 40; calcium pantothenate, 20; folic acid, 1; and biotin, 0.2. The diet was free of magnesium and devoid of any supplement of cholesterol or cholic acid. The diet as well as water was fed ad libitum. The animals were housed in individual cages with screened bottoms in a temperature-controlled room (72 F.). Venous blood was drawn approximately every month, and the serum analyzed for sodium and potassium by flame photometry. Electrocardiograms were taken while the dogs were awake and also while anesthetized with sodium pentobarbital (30 mg./Kg. I.V.).

Prior to being fed the experimental diet, all dogs were dewormed and vaccinated against distemper.

Experiment 2

The second experiment was started approximately three months after experiment 1. Ten mongrel puppies, approximately eight weeks old and weighing 4 to 6 Kg., were divided into two equal groups. one of which was fed the diet listed in experiment 1. The other group of five animals was fed the same diet, but 96 mg. of magnesium was added as MgO per 100 Gm. of diet. This level of magnesium was chosen, since previous studies on young growing rats showed that this amount was adequate for maximum rates of growth and resulted in minimal or no histopathological changes. The animals were housed and fed as in experiment 1. Venous blood was drawn, and the serum was analyzed for sodium and potassium by flame photometry, for cholesterol according to the method of Carpenter et al., and for serum proteins and lipoproteins by paper electrophoresis. The various chemical methods for deter-
Table 1

<table>
<thead>
<tr>
<th>Dogs</th>
<th>Weight (Kg.)</th>
<th>Days on experiment</th>
<th>Sodium (mEq./L.)</th>
<th>Potassium (mEq./L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(I/F)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.8/7.4</td>
<td>100</td>
<td>158/151</td>
<td>5.1/2.1</td>
</tr>
<tr>
<td>2</td>
<td>9.7/7.4</td>
<td>121</td>
<td>162/149</td>
<td>5.1/4.0</td>
</tr>
<tr>
<td>4</td>
<td>3.9/6.4</td>
<td>175</td>
<td>157/155</td>
<td>5.4/3.7</td>
</tr>
<tr>
<td>5</td>
<td>5.1/7.4</td>
<td>175</td>
<td>155/159</td>
<td>5.2/3.6</td>
</tr>
<tr>
<td>3*</td>
<td>6.8/17.2</td>
<td></td>
<td>156/157</td>
<td>5.4/4.1</td>
</tr>
<tr>
<td>6†</td>
<td>5.4/10.3</td>
<td></td>
<td>159/155</td>
<td>5.9/3.9</td>
</tr>
</tbody>
</table>

- Dog 3 convulsed within one month after being fed the deficient diet and was given a diet containing 24 mg. of magnesium during the remainder of the experiment.
- After approximately five months, dog 6 was fed a diet containing 24 mg. of magnesium per 100 Gm. for the remainder of the experiment. During this latter period he gained 3.0 Kg.
- I = initial weight at time of instituting experimental diet; F = final weight.
- All dogs were allowed to die from the deficiency; none was sacrificed. All were autopsied within 12 hours after death and usually within 2 hours.

Electrolyte concentrations are for serum, one to seven days prior to death.

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**Results**

It is clear from tables 1 and 2 that animals fed the magnesium-deficient diet grew less than did the control animals. The deficient animals gained a maximum of 2.5 Kg., whereas the controls gained 1.5 to 12.3 Kg. After about two months the deficient dogs appeared less well nourished clinically. Giving a deficient animal a diet containing magnesium (24 mg. per 100 Gm.) resulted in a prompt growth response after the animal had reached a weight plateau (dog 3) or had started to lose weight after a weight plateau (dog 6) (table 1).

Within one to two months the deficient animals became irritable when placed in the Pavlov stand, in contrast with the fairly placid control animals. All magnesium-deficient dogs convulsed several times during the course of the experiment. Three to five weeks before the animals became moribund, they developed a peculiar stance, illustrated in figure 1. There was extreme hyperflexibility and hyperextension of the front paws. There also appeared to be a hind-leg paralysis. Figure 1 illustrates a typical position taken by magnesium-deficient dogs when placed in the Pavlov stand.

Although the values are not tabulated here, serum calcium did not appear to be affected by magnesium deficiency nor did total serum protein, protein fractions, or lipoproteins. The serum-calcium levels were approximately 9 to 11 mg. per 100 cc. throughout the course of the experiment. Serum proteins varied between 5 and 6 Gm. per 100 cc.

Table 2 illustrates the great variability in serum-cholesterol levels of the mongrel pup-
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pies. There was no apparent effect of magnesium deficiency on serum-cholesterol concentrations.

Tables 1 and 2 show that magnesium deficiency also had no effect on the serum-sodium concentrations, which varied between 144 and 160 mEq./L. It did, however, have an effect on serum-potassium levels. Initially, these values were between 5 and 7 mEq./L. in both experiments. These initial serum-potassium values are within the range of that found in approximately 140 dogs fed Purina Laboratory Chow. After about 100 days on the diet, the serum potassium of dogs fed the magnesium-deficient diet had decreased an average of 2.0 mEq./L., while control animals showed an insignificant average decrease of less than 0.04 mEq./L. after the same length of time. Terminally, the average serum-magnesium level was 1.6 mg. per 100 cc. for control animals and 1.1 mg. per 100 cc. for deficient dogs. This difference in the serum-magnesium level between the two groups was not statistically significant.

There were electrocardiographic changes in both the control and deficient dogs. However, these changes were much more pronounced and more frequent in the deficient animals; thus their electrocardiograms could be readily differentiated from those of the controls. Figure 2 illustrates some of the changes seen in the electrocardiograms of magnesium-deficient dogs: peaking of the T waves, S-T-segment depression, and ready precipitation of sinus tachycardia with rates in excess of 200. The T-wave alterations were seen predominantly in the awake animal. Magnesium-deficient dogs also became increasingly sensitive to the cardiotoxic effects of digitalis. The dose required to produce ventricular tachycardia was decreased as the animals became more magnesium-deficient. The findings on the cardiotoxic effects of cardiac glycosides in control and deficient dogs, as well as the resting electrocardiograms, will be detailed in a later communication.

The histopathological findings in the magnesium-deficient dogs were clearly different in certain respects from those of the control dogs. In the deficient dogs calcification occurred in various portions of the cardiovascular system. Calcium was deposited near or upon the elastica of the inner portion of the aorta with a greater tendency for involvement of the internal elastica. Calcification of the aorta was grossly visible and was more pronounced in the thoracic than in the abdominal aorta. This deposition was upon the internal elastica of the coronary arteries and of medium-sized arteries in the greater circulation, e.g., arteries in various portions of the stomach, small and large intestines, kidneys, and pancreas. In all these structures calcium was laid down in the arterial media, sometimes eccentrically in both the longitudinal and horizontal axes.

In contrast to our experience with the rat on a low-magnesium diet for similar time periods, the magnesium-deficient dog appears to have at least a slight to moderate intimal reaction. The intimal thickening, which narrowed the arterial lumen of the coronary arteries by one-fourth at the most, consisted of loose connective tissue (fig. 3). Sometimes it was associated with moderate calcification of the media, but in a minority of sections the only visible calcification was a patchy one involving only the internal elastica. The intimal plaques and the interstitial tissue of the immediately underlying media frequently showed moderate metachromasia when stained with toluidine blue, while the media of control dogs.
arteries showed this characteristic in only slight degree at most. In frozen sections of coronary arteries, Sudan-IV-positive material was rarely found in the plaques and then only in small quantities. No fresh or organizing thrombi were encountered.

In severe cases, calcification of the arterial tree involved the small arteries of the spleen, the thymus, the capillaries of the lungs, and even the sinuses of the adrenals. Calcification of the small renal arteries was seen in every magnesium-deficient animal and was more severe in those animals having widespread and extensive calcification elsewhere. These calcified small renal arteries were frequently ectatic; such ectasia was found only in regions of calcification (fig. 4). Calcium deposition in the tubular lumens of the renal cortex was virtually absent in the deficient dogs. This contrasts to the marked renal tubular calcification in magnesium-deficient rats.

Another prominent finding in the magnesium-deficient dog was calcification of the heart. The calcification was most prominent in the inner portion of the myocardium of the left ventricle including the interventricular septum. The most extensively involved heart is shown in figure 5. The calcium was deposited in strips and wide bands; at the periphery of these regions the appearance suggested strongly that the calcinosis had involved individual muscle bundles. Between the regions of calcification there was loose connective tissue containing only few or no macrophages. In several calcified hearts the subendocardial elastica of the left atrium was the seat of calcinosis.

One control and two deficient dogs showed massive gastrointestinal bleeding with no ulcers but with large foci of gastric submucosal hemorrhages. Another control dog had peritonitis and a mild bronchopneumonia. Another deficient dog had an acute gangrenous enteritis and moderate bronchopneumonia. In all these animals, the histological appearance was that of an acute condition, and there was usually a terminal clinical worsening of the animal’s condition. The change or lack of change in electrolytes de-

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**Table 2**

<table>
<thead>
<tr>
<th>Dog number</th>
<th>Weight (kg) (mean)</th>
<th>Serum Cholesterol (mg/100 ml)</th>
<th>Calcium (mg/100 ml)</th>
<th>Magnesium (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.59 ± 0.11</td>
<td>3.2 ± 0.20</td>
<td>154 ± 19</td>
<td>1.1 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>3.69 ± 0.09</td>
<td>3.2 ± 0.20</td>
<td>154 ± 19</td>
<td>1.1 ± 0.12</td>
</tr>
<tr>
<td>3</td>
<td>3.66 ± 0.10</td>
<td>3.2 ± 0.20</td>
<td>154 ± 19</td>
<td>1.1 ± 0.12</td>
</tr>
</tbody>
</table>

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1. All dogs were allowed to die from the deficiency; none was sacrificed. All were autopsied within 12 hours after death and then within 2 hours.
2. The control dogs were fed a normal diet; the magnesium-deficient dogs were fed a diet containing magnesium deficient in magnesium-deficient rats.
3. The results are expressed as the mean ± standard deviation.
4. The statistical significance of the differences was determined by the Student's t-test.
5. Significant at P < 0.05.
6. Not significant at P > 0.05.
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Figure 2
Electrocardiograms (leads II and V₁) of puppy fed a magnesium-deficient diet for 120 days. Electrocardiograms in upper portion were taken at the beginning of the experiment; those in the lower portion at 120 days. Animal awake. Note peaking of T waves and slight depression of the S-T segment at 120 days.

scribed previously (see above) was not considered to be a function of these terminal complications, for: (a) only a minority of animals showed these complications; and (b) the electrolyte-change pattern determined periodically throughout the experiment clearly antedated these complications. Control dogs that had shown electrocardiographic changes showed no significant light microscopic changes in the heart. On the other hand, one magnesium-deficient dog with severe myocardial calcification had only minor electrocardiographic changes in contrast to the more extensive ones seen in other magnesium-deficient dogs with less myocardial calcification.

Discussion

There have been several studies that have stated that there is a correlation between serum magnesium and cholesterol. Bersohn and Oelofse¹¹ reported a significant inverse relationship between serum magnesium and cholesterol in population groups in South Africa. Malkiel-Shapiro et al.¹² reported clinical improvement in patients with coronary-artery disease with parenteral magnesium. Recently, Brown et al.¹³ failed to show any correlation between serum cholesterol and magnesium in clinically healthy American males and found no significant difference between serum magnesium in normal men and in those with myocardial infarction. Charnock et al.¹⁴ also failed to show any correlation between serum cholesterol and magnesium concentrations in the central Australian aborigine or in Europeans with or without ischemic heart disease. However, these same authors showed that the aborigines had a significantly lower serum-cholesterol level and a significantly higher serum-magnesium level than did the Europeans. They attributed these differences in part to dietary habits. In the present study, magnesium deficiency appeared to exert no effect on serum-cholesterol levels.

While the weight of evidence suggests that there is no relationship between serum magnesium and cholesterol, it is possible that both may affect some other factor(s) involved in lipid metabolism. High dietary magnesium has been shown to protect against vascular lipidosis in cholesterol- and cholic acid-fed rats without affecting the serum-cholesterol level.³ ⁴ Thus far, attempts to promote atheromatosis in dogs simply by feeding cholesterol have not met with much success.¹⁵ While no atheromatosis was seen in any of the animals in the present study, it is possible that feeding cholesterol to magnesium-deficient dogs might
result in lipid infiltration of the intimal fibrous plaques seen.

The calcification of the inner portion of the left ventricular myocardium is not attributed to the changes in the coronary arteries. One explanation of the location of this calcification would be that the outer myocardium had first access to the coronary arterial blood flow and to the limited serum ions thereof and was, therefore, less deficient in nutrients than was the inner myocardium. A second possibility would be that the inner myocardial cells are to some degree functionally and biochemically different from the outer ones and, therefore, respond differently—e.g., more readily—to a deficiency. Were this latter consideration to have some validity, it would be relevant to the fact that with human coronary-artery thrombosis the degree of myocardial infarction is greater in the inner portion of the myocardium than in the outer portion.

Syllm-Rapoport and Strassburger reported that a dog on a magnesium-deficient diet showed no calcification, while three other such dogs that additionally had received intravenous calcium chloride injection exhibited macroscopic calcification of heart, aorta, arteries, and kidney. The dogs in our experiments received no calcium injection. The experimental period of our deficient dogs was appreciably longer than the apparent six to seven weeks of their experiment; also, microscopic examination was not mentioned in their report. These factors may explain the differences between their and our results.

On the basis of data from magnesium-deficient rats and on a priori reasoning, one might expect that magnesium-deficient dogs would have a lowered serum magnesium. Kruse and co-workers found that the serum magnesium of the two deficient dogs on a magnesium-deficient diet dropped early in the course of the experiment, whereas no changes in the blood calcium or phosphorus were noted; blood potassium was not studied. In contrast, in the present experiment, the serum magnesium of the dogs fed the magnesium-deficient diet was lower than that of the control animals, but this difference was not statistically significant (P > 0.05).

As was shown in tables 1 and 2, dogs fed a magnesium-deficient diet developed a lowered serum-potassium concentration in contrast with control dogs, which developed no such changes. MacIntyre and Davidsson found that magnesium deficiency in rats produced a secondary intracellular potassium deficiency, a slight decrease in the serum potassium, and a significant rise in the serum sodium. Peaking of the T waves, which was observed in the dogs fed the magnesium-deficient diet in the present experiments, was similar to that seen in the presence of hyper-
kalemia. This peaking, however, occurred in dogs on the deficient diet, dogs with a reduced serum-potassium concentration. Since with a reduced extracellular potassium there is a cellular deficit of this cation, magnesium deficiency may produce a relative hyperkalemia; that is, a greater decrease in intracellular than in extracellular potassium levels. It remains to be proved that there is indeed a decrease in intracellular potassium in dogs fed a magnesium-deficient diet.

It has been shown that magnesium is essential for oxidative phosphorylation and that there is an uncoupling of this reaction in mitochondria isolated from magnesium-deficient rats. Various studies have demonstrated that intact, viable mitochondria with ability to carry out oxidative phosphorylation are a requisite for maintaining sodium- and potassium-concentration gradients. Thus, the electrocardiographic changes seen in the deficient dogs may be the result of intracellular losses of potassium and, perhaps, of magnesium as well.

Summary

Diet-induced magnesium deficiency in male puppies resulted in lowered weight gain, hyperrirritability, and convulsions. The histopathological changes consisted of calcification of the elastica and media of the aorta, of coronary and other peripheral arteries, and of the inner portion of the myocardium. Fibrous, virtually fat-free, plaques were seen in the coronary arteries. Magnesium deficiency had no effect on serum cholesterol, sodium, calcium, or total protein. Slight lowering of serum magnesium lacked statistical significance. There was a significant decrease in the serum-potassium concentration of these magnesium-deficient dogs. Electrocardiographic changes were noted.

Acknowledgment

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References


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